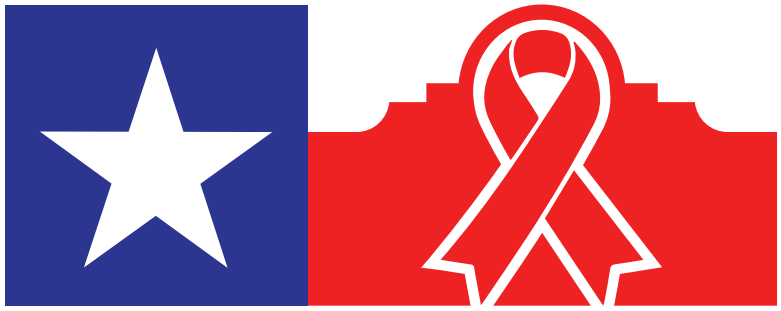

Nonhuman Primate Models for AIDS

37th Annual Symposium
San Antonio 2019

November 12-15, 2019
Omni La Mansion del Rio Hotel
San Antonio Texas

Hosted by

TEXAS BIOMEDICAL RESEARCH INSTITUTE

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The 38th Annual Symposium on Nonhuman Primate Models for AIDS

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Portland, Oregon

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Oregon Health & Science University

Venue Portland Marriott Downtown Waterfront Hotel

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SAN ANTONIO 2019
Nonhuman Primate Models for AIDS
37th Annual Symposium

Welcome to the 37th Annual Symposium on Nonhuman Primate Models for AIDS, held at the Omni La Mansion del Rio Hotel in downtown San Antonio. The principal objective of this symposium is to serve as a scientific forum for the dissemination and exchange of new research findings, ideas and directions by an international group of scientists whose research focuses on the study of natural or experimental immunodeficiency virus infections, i.e., Human Immunodeficiency Virus (HIV), Simian Immunodeficiency Virus (SIV) and recombinant Simian/Human Immunodeficiency Virus (SHIV), in nonhuman primates. The ultimate goal is to utilize the knowledge gained from these crucial nonhuman primate studies to better understand how HIV and SIV cause disease in order to facilitate the development of new methods for the treatment, control and prevention of AIDS in human populations.

The Annual Symposium on Nonhuman Primate Models for AIDS had its origin at the Delta Regional Primate Research Center in December 1983 when approximately 30 pathologists, virologists, immunologists and clinical veterinarians from the seven Regional Primate Research Centers met to discuss what was then a poorly understood, spontaneously occurring immunodeficiency syndrome of macaque monkeys that had been recognized at several of the Centers since 1969. Although its cause at that time was not known, the disease had many clinical and pathologic similarities to the newly emerging human epidemic, acquired immunodeficiency syndrome, or AIDS, first described in 1981.

We are excited to welcome you to this year's symposium in San Antonio, Texas. The Alamo City, as it is often called, celebrated its Tricentennial birthday in 2018. Over its 300-year history, the city has become the seventh largest city in America. Healthcare and biosciences are the dominant force in the city's economy, serving as the leading industry employing more than 1 of every 6 members of the San Antonio workforce. As one local organization, BiomedSA, attests, "The sector combines unique assets, diverse resources, and a tangible spirit of collaboration." It is in this spirit of collaboration that the Southwest National Primate Research Center host this year's symposium.

In 1999, the Southwest National Primate Research Center (SNPRC) became the first new NCRR-funded National Primate Research Center (NPRC) in over 35 years. Texas Biomedical Research Institute, the host institution for the SNPRC, began to establish its nonhuman primate facilities and resources in 1958 and has a long tradition of biomedical and behavioral research with nonhuman primates. Texas Biomed hosted the 6th Annual Symposium in 1988, the 22nd in 2004, and the 30th in 2012. Now, coinciding with the 20th anniversary of the SNPRC, it is our honor to welcome you to the 37th edition of this important meeting.

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Acknowledgments

As researchers, we are focused on preventing the spread of AIDS. Our annual meeting is the leading forum for the exchange of the latest developments in AIDS research using nonhuman primates. This forum attracts more than 200 of the world's foremost researchers to share their findings and ideas for preventing the spread of AIDS. Findings and outcomes from this symposium will have a global impact.

Texas Biomedical Research Institute and the Southwest National Primate Research Center are proud to host the 37th Annual Symposium on Nonhuman Primate Models for AIDS in 2019, which will be held at the beautiful Omni La Mansion del Rio Hotel in San Antonio, TX. The NHP Symposium has a long-standing tradition of playing a vital role in disseminating the most current and relevant findings in both basic and applied areas of AIDS research. The conference typically attracts more than 200 leading scientists in many areas of AIDS-related research, representing renowned universities, research institutes and corporations worldwide.

This symposium will be funded in part by a grant from the Office of the Director of the National Institutes of Health (NIH), as well as attendee registration fees. Contributions from private and corporate sponsors, however, will have enabled us to maintain the high quality of programming and networking opportunities.

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General Information

Welcome to the 37th Annual NHP Models for AIDS Symposium in San Antonio, Texas. We look forward to a great event. Below are some logistical details to keep in mind throughout the event. If you have any questions at all during the event, please reach out to one of the co-chairs or visit the registration table in the lobby of the Omni La Mansion del Rio between noon-9pm Tuesday, November 12 or between 7am-5pm Wednesday, November 13.

The symposium will consist of a welcome night Keynote Speaker, Dr. Mario Roeder, at 6 pm in the Iberian Ballroom followed by a reception in the Madero Ballroom on Tuesday, November 12.

Five Scientific Sessions (all held in the Iberian Ballroom) begin Wednesday, November 13 and run through noon on Friday, November 15. A Poster session (in the Veramendi Room) will be held Wednesday evening, November 13 and our closing Banquet Reception will be held at the Briscoe Western Art Museum from 5:15-10 pm with special activities to include river barge rides and museum entrance that evening.

Each of the five scientific sessions has two co-chairs, co-chairs will be responsible for introducing speakers and recognizing questioners at the end of each presentation. In addition, the Session Chairs have been asked to give 15 to 30-minute presentations, summarizing critical developments in that particular area of research and their own contributions.

Other selected oral presentations will be 10 minutes long with five minutes reserved for questions from the audience. These presentations were selected by members of the Scientific Program Committee, which reviewed abstracts and scored submissions in a blind fashion.

Scientific Session I: Biology of Primate Lentivirus Infections

Chairs: W. Johnson and M. Mohan

Scientific Session II: Pathogenesis of Primate Lentivirus Infections

Chairs: D. Evans and M. Muller-Trutwin

Scientific Session III: Co-infections in the NHP Model

Chairs: M. Kuroda and D. Kaushal

Scientific Session IV: Therapeutics/Cure in the NHP Model

Chairs: S. Valente and L. Giavedoni

Scientific Session V: Vaccine Development

Chairs: M. Ackerman and M.C. Gauduin

Important: Please bring a copy of your presentation on removable media (flash drive) to the Registration Desk at the Welcome Reception on Tuesday night or during the break before your presentation time. We will upload your presentation onto conference computers and review briefly with you. Presentations will be driven by laptop computers.

Mid-morning and mid-afternoon breaks in the Madero Ballroom will be included within these half-day sessions to facilitate informal exchange of ideas among conferees.

For poster presenters, there are several opportunities for you to hang your poster before the Poster Reception Wednesday evening, November 13. Please have your poster hung before noon on Wednesday. Posters must be removed by noon on Thursday, November 14.

Each guest will be provided two drink tickets for each of the events: Welcome Reception, Poster Reception and Banquet Dinner. Please present these to receive complimentary beer, wine or cocktail. A cash bar will also be available at each event.

WiFi is provided through the hotel.

1. Connect to “Omni Meeting” WIFI
2. Open a web browser and enter the following information into the Omni splash page.
3. Access Code: NHP2019

Award Recipients

RECIPIENTS OF EARLY INVESTIGATOR AWARDS:

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Ann Marie Carias

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Justin Green

Welcome Event Keynote Speaker

Mario Roederer, PhD

"Developing the NHP AIDS model for antibody-based interventions"

Dr. Roederer received his B.S. in chemistry in 1983 from Harvey Mudd College, Claremont, California, followed by his Ph.D. in biological sciences in 1988 from Carnegie Mellon, Pittsburgh, in the laboratory of Dr. Robert Murphy. He trained as a postdoctoral fellow and then as a research fellow at Stanford University from 1988 to 1999 in the laboratory of Dr. Leonard Herzenberg. Following this, he was adjunct associate professor, department of Stomatology, University of California, San Francisco, until 2000, when he joined the Vaccine Research Center (VRC) at NIH. He is a senior investigator and is Chief of ImmunoTechnology Section, director of the Flow Cytometry Core, and director of the Nonhuman Primate Immunogenicity Core. Dr. Roederer has co-authored six patents, five software titles in worldwide use, and more than 300 papers with over 48,000 citations. The choice of Dr. Roederer as Keynote Speaker is in line with the theme of the meeting; his research combines cutting-edge technology development in the setting of single cell analysis (integrating both flow cytometry and transcriptomics), with basic T and B cell immunology. Over the past two decades, he led the effort to the development of the state-of-the-art 30+ color flow cytometry, and has produced several seminal publications demonstrating the highly translational value of the rhesus macaque/SIV model for AIDS research. Basic research projects include T cell dynamics during HIV or SIV infection; the definition of the complete repertoire of functions elicited by vaccines and pathogens; characterization of memory stem cells for generation and maintenance of long-term T cell memory; mucosal delivery of vaccines for use in influenza, RSV, and TB; and understanding the antigenic heterogeneity of HIV/SIV Env to develop protective antibodies.

Banquet Speaker

Alan Schultz, PhD

“Impact of NHP Model(s) on AIDS Vaccines – and vice versa”

Dr. Schultz received an undergraduate degree in Chemistry from the University of Rochester, a Ph.D. in Biology from the Johns Hopkins University, and later worked in Dr. Robert Gallo’s laboratory, where he was introduced to the field of retrovirology. More recently, he completed 30 years in AIDS vaccine development, the first 10 years of which were at the Division of AIDS, devoted to stimulating and fostering NHP models for AIDS and their application to vaccine development and forging the initial funding structures to provide money to the extramural community. There followed ten years at IAVI utilizing NHP for translational and developmental research, capped with a final 10 years returning to DAIDS to continue encouraging and facilitating vaccine research and challenge studies in NHP.

The foundation for this history was postdoctoral studies on SV40 and then the stint in the Gallo laboratory, further refined by 11 years at NCI-Frederick studying the viral proteins and oncoproteins of retroviruses (of mice, cats, cows, primates and humans) and their post-translational modifications in Stephen Oroszlan’s laboratory, which did the first amino acid sequence from an AIDS virus protein. It was frustration that SIV was initially being ignored that led him to leave Frederick and join the newly-formed NIAID AIDS Program in 1988.

Agenda

TUESDAY, NOVEMBER 12, 2019

12:00pm – 9:00pm	**Registration** Omni La Mansion Hotel Lobby
3:00pm – 9:00pm	**Poster Set up** Omni La Mansion Veramendi Room
6:00pm	**Welcome Address** Larry Schlesinger, M.D. Texas Biomedical Research Institute Omni La Mansion Iberian Ballroom
6:10pm – 7:00pm	**Keynote Speaker: Developing the NHP AIDS model for antibody-based interventions** Mario Roederer, Ph.D. Omni La Mansion Iberian Ballroom
7:00pm – 9:00pm	**Welcome Reception** Omni La Mansion Madero Room

WEDNESDAY, NOVEMBER 13, 2019

7:00am – 3:00pm	**Registration** Omni La Mansion Hotel Lobby
7:00am – 12:00pm	**Poster Set up** Omni La Mansion Veramendi Room
7:00am – 8:30am	**Continental Breakfast** Omni La Mansion Madero Ballroom
8:00am – 5:00pm	**Sponsor Displays** Omni La Mansion Madero Ballroom

SESSION I: BIOLOGY OF PRIMATE LENTIVIRUS INFECTIONS
Session Co-Chairs: Welkin Johnson and Mahesh Mohan

8:30am - 9:00am	**Evolution of the capsid-host interactions and adaptation of primate lentiviruses to spillover hosts** Welkin Johnson (Session Co-Chair)
9:00am – 10:00am	**Session I Presentations**
9:00am – 9:15am	**Abstract 1: A glycan shield on chimpanzee CD4 protects against infection by primate lentiviruses (HIV/SIV)** Cody Warren
9:15am – 9:30am	**Abstract 2:** **Mucosal perturbation of natural killer cell receptor-expressing B cells in lentivirus infected primates** Cordelia Manickam
9:30am – 9:45am	**Abstract 3:** **Temporal and spatial characterization of SIV infection dynamics in rhesus macaque mucosal tissues.** Danijela Maric
9:45am – 10:00am	**Abstract 4:** **APOBEC3 diversity and its role in hypermutation of human and simian immunodeficiency viruses** Diako Ebrahimi
10:00am – 10:30am	**Coffee Break- Omni La Mansion Madero Ballroom**
10:30am – 12:00pm	**Session I Presentations Continued**
10:30am – 10:45am	**Abstract 5:** **Mimicking SIV chimpanzee viral evolution towards HIV-1 during cross-species transmission** Kimberly Schmitt
10:45am – 11:00am	**Abstract 6:** **Baboon Model of Innate Immunity against Immunodeficiency Virus Infection** Amanda Mannino
11:00am – 11:15am	**Abstract 7:** **SERINC5 Antagonism is Separable from other Nef Functions** Sanath Kumar Janaka
11:15am – 11:30am	**Abstract 8:** **Th17-cells are early targets of SIV-infection during acute infection in Rhesus Macaque Model** Muhammad Shoaib Arif

11:30am – 11:45am	**Abstract 9:** **Identification and characterization of CD94 (KLRD1)+ memory-like NK cells in rhCMV- and SIV-infected rhesus macaques using RNA-flow cytometry** Daniel Ram
11:45am – 12:00pm	**Abstract 10:** **The landscape of immune responses and persistent viral reservoirs in systemic and lymphoid tissues in rhesus macaques infected by a newly developed SHIV-CH848** Widade Ziani
12:00pm – 1:30pm	**Lunch on your own**

SESSION II: PATHOGENESIS OF PRIMATE LENTIVIRUS INFECTIONS
Session Co-Chairs: Michaela Muller-Trutwin and David Evans

1:30pm – 2:00pm	**Tissue-specific viral control in a natural host of SIV** Michaela Muller-Trutwin (Session Co-Chair)
2:00pm – 3:00pm	**Session II Presentations**
2:00pm – 2:15pm	**Abstract 11:** **Adipose tissue contributes to viral persistence in ART-treated SIV.sab infection in pigtailed macaques** Paola Sette
2:15pm – 2:30pm	**Abstract 12:** **Rapid progression in SIV-infected infant rhesus macaques is associated with failure to increase CXCL13 during acute infection, B cell dysfunction and early viral replication** Matthew Wood
2:30pm – 2:45pm	**Abstract 13:** **Modulation of host responses to HIV infection by long non-coding RNAs** Smita Kulkarni
2:45pm – 3:00pm	**Abstract 14:** **Chronic morphine administration affects SIV reservoirs** Arpan Acharya
3:00pm – 3:30pm	**Coffee Break- Omni La Mansion Madero Ballroom**

3:30pm – 5:00pm	**Session II Presentations Continued**
3:30pm – 4:00pm	**Resistance of HIV- and SIV-infected cells to elimination by antibodies** David Evans (Session Co-Chair)
4:00pm – 4:15pm	**Abstract 15:** **Monocyte turnover increase reflects tissue macrophage destruction and disease pro-gression to AIDS in SIV-infected rhesus macaques.** Naofumi Takahashi
4:15pm – 4:30pm	**Abstract 16:** **Long term delta-9-tetrahydrocannabinol administration inhibits oral mucosal inflammation in chronically SIV-infected rhesus macaques through regulating microRNA mediated pathways and the oral microbiome** Mahesh Mohan (Session Co-Chair)
4:30pm – 4:45pm	**Abstract 17:** **Functional perturbation of mucosal group 3 innate lymphoid and natural killer cells in SHIV.CH505-infected neonatal rhesus macaques** Brady Hueber
4:45pm – 5:00pm	**Abstract 18:** **Enhanced Protection at the Site of Challenge of Rhesus Macaques That Receive PGT121 One Week Prior to Intravaginal Challenge With SHIV-SF162P3** Jeffrey Schneider
6:00pm – 9:00pm	**Poster Reception** Omni La Mansion Veramendi Room

THURSDAY, NOVEMBER 14, 2019

7:00am – 8:30am	**Continental Breakfast** Omni La Mansion Madero Ballroom
8:00am – 5:00pm	**Sponsor Displays** Omni La Mansion Madero Ballroom

SESSION III: CO-INFECTIONS IN THE NHP MODEL
Session Co-Chairs: Marcelo Kuroda and Deepak Kaushal

8:30am - 9:00am	**Macrophage damage by SIV is critical for TB reactivation and AIDS disease progression in rhesus macaques** Marcelo Kuroda (Session Co-Chair)
9:00am – 10:00am	**Session III Presentations**
9:00am – 9:15am	**Abstract 19:** **Chronic immune activation due to SIV co-infection correlates with reactivation of latent tuberculosis infection** Allison Bucsan
9:15am – 9:30am	**Abstract 20:** **A Non-human Primate Model for Vaginally Acquired Syphilis** Sundaram Ajay Vishwanathan
9:30am – 9:45am	**Abstract 21:** **Characterization of MAIT cells in Mauritian cynomolgus macaques (MCM) SIV/Mtb co-infection** Alexis Balgeman
9:45am – 10:00am	**Abstract 22:** **Rhesus Macaques Support Chronic HBV infection in vivo and are an emerging model of HIV/HBV co-infection** Sreya Biswas
10:00am – 10:30am	Coffee Break- Omni La Mansion Madero Ballroom
10:30am – 12:00pm	**Session III Presentations Continued**
10:30am – 10:45am	**HIV and TB: New Approaches to Treating the Syndemic** Deepak Kaushal (Session Co-Chair)
10:45am – 11:00am	**Abstract 23:** **Fetal brain transcriptomic signature of congenital Zika syndrome identifies alterations in the myelination process** Jennifer Tisoncik-Go
11:00am – 11:15am	**Abstract 24:** **Early induction of Mycobacterial lipid antigen-specific responses in BCG-inoculated macaques and impact of SIV coinfection on these responses** Namita Rout
11:15am – 11:30am	**Abstract 25:** **Impact of P. fragile infection on SHIV.CH505 acquisition and viral load in rhesus macaques** Jennifer Manuzak

11:30am – 11:45am	**Abstract 26:** **Maintenance of Mtb antigen specific CXCR3 and CCR6 co-expressing cells in BAL are associated with immune control in LTBI Rhesus Macaques** Uma Shanmugasundaram
11:45am – 12:00pm	**Abstract 27:** **Can cART alone deliver in the race between immune reconstitution and TB reactivation?** Shashank Ganatra
12:00pm – 1:30pm	**Lunch on your own**

SESSION IV: THERAPEUTICS/CURE IN THE NHP MODEL
Session Co-Chairs: Susana Valente and Luis Giavedoni

1:30pm – 2:00pm	**Silencing the HIV-1 reservoir, the "Block and Lock" approach** Susana Valente (Session Co-Chair)
2:00pm – 3:00pm	**Session IV Presentations**
2:00pm – 2:15pm	**Abstract 28:** **CD4-MBL-CAR/CXCR5 T cells suppress SIV replication post-ART release** Pamela J. Skinner
2:15pm – 2:30pm	**Abstract 29:** **Distribution of I.V. injected Cu64, Zr89, and fluorescently labeled VRC01 and VRC01-LS in the in vivo Rhesus Macaque Model** Ann Marie Carias
2:30pm – 2:45pm	**Abstract 30:** **Viral & Immune Dynamics in an NHP Model of Pediatric HIV Infection: Studies to Inform Cure Strategies** Veronica Obregon-Perko
2:45pm – 3:00pm	**Abstract 31:** **HIV-Specific Chimeric Antigen Receptor T-Cells Expand and Persist Following Antigen Boost in Infected, Suppressed Rhesus Macaques** Christopher Peterson
3:00pm – 3:30pm	**Coffee Break- Omni La Mansion Madero Ballroom**
3:30pm – 5:00pm	**Session IV Presentations Continued**
3:30pm – 3:45pm	**Inactivation of proviral SIV DNA with different forms of Cas9** Luis Giavedoni (Session Co-Chair)

3:45pm – 4:00pm	**Abstract 32:** **Single dose post-exposure treatment with bNAb cocktail achieves clearance of SHIV in infant macaques** Ann Hessell
4:00pm – 4:15pm	**Abstract 33:** **Directed homing of SIV-specific TCR-engineered T cells to B cell follicles through ectopic expression of CXCR5** Daniel Burke
4:15pm – 4:30pm	**Abstract 34:** **CD8+ T cells provide limited control of SIV reactivation after combination antiretroviral therapy (cART) cessation** Afam Okoye
4:30pm – 4:45pm	**Abstract 35:** **Virologic response to concomitant administration of SIV-specific engineered T cells and heterodimeric IL-15 in a SIV-infected Rhesus macaque** Brenna Hill
4:45pm – 5:00pm	**Abstract 36:** **Control of virus replication in SIV-infected rhesus macaques after therapeutic vaccination is associated with polyfunctional SIV specific T-cells localized in the mesenteric lymph nodes.** Hillary C. Tunggal
5:15-6:15pm	**River Barge Transportation to Banquet at Briscoe Museum**
5:30-7:30pm	**Briscoe Museum open for attendees**
6:00pm – 10:00pm	**Banquet:** **Speaker Alan Schulz, Ph.D.** **"Impact of NHP Model(s) on AIDS Vaccines - and vice versa"** Briscoe Western Art Museum's Guenther Pavilion

FRIDAY, NOVEMBER 15, 2019

7:00am – 8:30am	**Continental Breakfast** Omni La Mansion Madero Ballroom

SESSION V: VACCINE DEVELOPMENT
Session Co-Chairs: Margareth Ackerman and Marie-Claire Gauduin

8:30am - 9:00am	**Learning important immune connections: correlates of protection across studies and species Margareth Ackerman (Session Co-Chair)**
9:00am – 10:00am	**Session V Presentations**
9:00am – 9:15am	**Abstract 37:** **Single-cell cloning of natural killer cells for in depth analysis of adaptive functions against HIV/SIV** Olivier Lucar
9:15am – 9:30am	**Abstract 38:** **Protective gene expression signature of the RhCMV/SIV vaccine** Fredrik Barrenas
9:30am – 9:45am	**Abstract 39:** **Rhesus macaques co-immunized with influenza and SIV DNA vaccines develop similar responsiveness to both vaccines** Sandra Dross
9:45am – 10:00am	**Abstract 40:** **Adjuvant-dependent Modulation of CD4 T Follicular Helper Cells Impacts Longevity and Functional Quality of Antibody Responses to a subtype C HIV-1 Envelope Vaccine in Rhesus Macaques** Smita S. Iyer
10:00am – 10:30pm	**Coffee Break- Omni La Mansion Madero Ballroom**
10:30am – 12:00pm	**Session V Presentations Continued**
10:30am – 10:45am	**Epithelial Stem Cell-based AIDS Vaccine to Induce Mucosal Immune Responses and Protection against SIV Challenge in Macaques** Marie-Claire Gauduin (Session Co-chair)
10:45am – 11:00am	**Abstract 41:** **A vaccination regimen to enhance immune responses associated with reduced risk of mother-to-child transmission of HIV** Ashley Nelson
11:00am – 11:15am	**Abstract 42:** **CMV vaccine vector-induced protection against SIV in Mauritian cynomolgus macaques** Justin Greene
11:15am – 11:30am	**Abstract 43:** **Contribution of vaccine-induced mutant epitope-crossreactive CD8+ T cells to stable control of SIVmac239 replication** Takushi Nomura

11:30am – 11:45am	**Abstract 44:** **Efficacy of Novel Recombinant Simian Varicella Virus-Simian Immunodeficiency Vaccines, rSVV-SIV, Following Mucosal SIV Challenge in the Rhesus Macaque** Vicki Traina-Dorge
11:45am – 12:00pm	**Abstract 45:** **Sequence and structure guided HIV-1 Clade C trimeric immunogen design to induce neutralizing and V1V2 directed antibody responses** Anusmita Sahoo
12:00pm – 12:15pm	**Closing remarks**

Abstracts

Session I:
Biology of Primate Lentivirus Infections

Co-Chair: Dr. Welkin Johnson

Evolution of the capsid-host interactions and adaptation of primate lentiviruses to spillover hosts

The icosahedral capsid cores of primate lentiviruses comprise thousands of copies of the viral capsid protein (CA). Upon entry into the host-cell cytoplasm, the capsid core engages in multiple, post-entry interactions with host factors that influence downstream steps in the replication cycle, and which ultimately impact the outcome of infection. Among these interactors are three cellular factors with identical or very closely overlapping interaction sites on the viral capsid core: these are 1) cyclophilin-A (CypA), 2) TRIM5alpha/TRIMcyp, and 3) the nuclear pore complex protein Nup358. Comparative analysis of human and nonhuman primate lentiviruses in the context of interspecies transmission and emergence reveals a complex evolutionary interplay between viral capsid proteins and these three factors, and provides insight into the nature of the interactions and their potential biological relevance. For example, we have established that TRIM5-mediated restriction in cell-culture correlates strongly with viral loads in SIVsm-infected macaques, explains a significant fraction of the variance in viral loads, and selects for emergence of resistance mutations in the surface domains of CA. Conversely, we found evidence that primate lentiviruses have driven evolution of the TRIM5 locus in old world primates, selecting for lentivirus-specific modifications in the TRIM5 SPRY domain. We've also established that the interaction between CA and the Nup358-Cyp domain is widely conserved among primate lentiviruses, is maintained by natural selection, and can be genetically distinguished from CA-CypA interactions. Thus, we argue that the interaction with Nup358 must be biologically relevant and selectively advantageous to lentiviruses in vivo. SIVmac, which is widely used to infect macaques as a model for studying HIV-1 infection and AIDS, proves to be an interesting exception, as it does not interact with Nup358-Cyp – we propose that this reflects an evolutionary trade-off that allowed escape from macaque TRIM5-Cyp alleles during emergence of SIVmac in NPRC colonies. Whether this results in unique outcomes in the SIVmac/macaque model remains to be seen. Going forward, introducing naturally occurring amino-acid variants into cloned viral proteins (e.g., in SIV CA) and into cloned primate host proteins (e.g. NUP358 & TRIM5 homologs) provides an elegant means by which to define the relevance of these interactions using molecular/biochemical assays.

1

A glycan shield on chimpanzee CD4 protects against infection by primate lentiviruses (HIV/SIV)

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Pandemic HIV-1 (group M) emerged following the cross-species transmission of a simian immunodeficiency virus from chimpanzees (SIVcpz) to humans. Primate lentiviruses (HIV/SIV) require the T cell receptor CD4 to enter into target cells. By surveying the sequence and function of CD4 in 50 chimpanzee individuals, we find that all chimpanzee CD4 alleles encode a fixed, chimpanzee-specific substitution (34T) that creates a glycosylation site on the virus binding surface of the CD4 receptor. Additionally, a single nucleotide polymorphism has arisen in chimpanzee CD4 (68T) that creates a second glycosylation site on the same virus binding interface. This substitution is not yet fixed, but instead alleles containing this SNP are still circulating within chimpanzee populations. Thus, all allelic versions of chimpanzee CD4 are singly glycosylated at the virus binding surface, and some allelic versions are doubly glycosylated. Doubly glycosylated forms of chimpanzee CD4 reduce HIV-1 and SIVcpz infection by as much as two orders of magnitude. Full restoration of virus infection in cells bearing chimpanzee CD4 requires reversion of both threonines at sites 34 and 68, destroying both of the glycosylation sites, suggesting that the effects of the glycans are additive. Differentially glycosylated CD4 receptors were biochemically purified and used in neutralization assays and microscale thermophoresis to show that the glycans on chimpanzee CD4 reduce binding affinity with the lentiviral surface glycoprotein, Env. These glycans create a shield that protects CD4 from being engaged by viruses, demonstrating a powerful new form of host resistance against deadly primate lentiviruses. Further, this glycan shield likely compromises experimental HIV-1 infection in at least some chimpanzees.

2

Mucosal perturbation of natural killer cell receptor-expressing B cells in lentivirus infected primates

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Objective: Recently, a novel innate immune cell subset bearing features of natural killer and B cells was identified in mice. So-called NKB cells appear as first responders to infections, but whether this cell population is truly novel or is in fact a subpopulation of B cells and exists in higher primates remained unclear. In this study, we identified and characterized NKBs for phenotype, trafficking and signalling in rhesus macaques after SHIV/SIV infection. Methods: NKB cells were quantified in both naïve and lentivirus-infected humans, neonatal and adult rhesus macaques by excluding lineage markers (CD3, CD127), and positive Boolean gating for CD20, NKG2A/C and/or NKp46 using traditional flow cytometry. Additional phenotypic measures were conducted by imaging cytometry using Amnis Image stream and multiparametric data analysis by t-distributed stochastic neighborhood embedding (tSNE). Results: Imaging cytometry confirmed $CD3^{-}CD20^{+}NKG2A^{+}$ as a unique phenotype of the NKB subset with no contamination from NK and B cell subsets and in addition, NKB cells clustered separately from B cell and NK cell clusters when analyzed by tSNE. Circulating NKB cells were found at similar frequencies in humans and adult rhesus macaques (range, 0.01 to 0.2% of total lymphocytes cells) and expressed surface IgA, IgM and IgG; trafficking marker CD62L, signalling molecules SLAM and Syk. While NKBs expanded in colon of SIV infected adult macaqes, a reduced frequency of NKB was observed in the colon of SIV/SHIV-infected neonatal macaques indicating differential perturbation of the NKB cell subset by lentiviruses. Conclusion: These results suggest a cell type expressing both NK and B cell features exists in both rhesus macaques and humans and are perturbed by lentivirus even as early as few months after birth. Their development and full functional niche remain unknown, but the unique phenotype and mucosal distribution could make NKB cells unique targets for immunotherapeutics or vaccine strategies.

3

Temporal and spatial characterization of SIV infection dynamics in rhesus macaque mucosal tissues.

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Our preliminary work revealed that Th17 T helper cells and immature dendritic cells are the most predominant initial targets after rectal challenge with an SIV-based replication defective reporter virus. These cell types are infected at a rate that is several-fold higher than their relative abundance predicts, indicating that they are preferentially targeted in the early time of infection. Here, we challenged twelve female macaques with a mixture of replication defective luciferase reporter and wild-type SIVmac239 and we sacrificed the animals 48-, 72- or 96h later. We used luciferase signal to home in on small regions within the tissue to increase our chances of identifying cells infected by wild-type virus. Infected cells were identified microscopically by staining for SIV viral proteins and were validated by spectral imaging and nested PCR. Foci of infected cells are visible as early as 48-hour post challenge and expand in size by 96 hours, stretching over several 10-micron tissue sections at times. Analysis of SIV infected cells revealed expected virus induced changes in CD4 expression, including CD4 receptor internalization and down-regulation. Comprehensive phenotypic profiling of nearly 2,000 SIV infected cells revealed that the Th17 infection rate does not vary much over the first 96h. However, from 48h to 96h, there is a pronounced decrease in iDCs infection rate and an increase in infection of other T cell subtypes, suggesting immune cell recruitment to the site of infection. Using the wild-type SIVmac239 virus we were able to study the early infection events at the rectal mucosa and we observed very dynamic changes in respect to infected cell phenotype and immune cell recruitment in response to infection. In our future work we hope to paint the full picture of the HIV/SIV sexual transmission in time and in space and hence aid development of more effective HIV prevention strategies.

4

APOBEC3 diversity and its role in hypermutation of human and simian immunodeficiency viruses

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The genomes of humans and non-human primates contain multiple APOBEC3 genes. These genes code for DNA cytosine deaminase enzymes, some of which are active against retroviruses, particularly HIV and SIV. These restrictive cytosine deaminase enzymes are able to induce lethal levels of C-to-U mutations (G-to-A mutation in the positive strand) in retroviral DNA during reverse transcription. Polymorphism in the APOBEC3 locus has been linked to differential antiretroviral immunity. We have discovered that HIV-1 sequences obtained from different HIV-1 patients as well as SIV infected non-human primates exhibit widely different levels and patterns of APOBEC3-induced hypermutation. Our combined computational/experimental analyses suggest that APOBEC3H diversity at both genomic and transcriptomic levels may play a significant role in the observed differential hypermutation patters.

5

Mimicking SIV chimpanzee viral evolution towards HIV-1 during cross-species transmission

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The Human Immunodeficiency Virus Type 1 (HIV-1) is believed to have arisen through cross-species transmission of simian immunodeficiency viruses (SIV) native to chimpanzees and gorillas. How the progenitor virus evolved into HIV-1 through genetic changes is not well understood. Previous studies have attempted to model this evolution using humanized mice (hu-mice). Hu-mice transplanted with human HSC (hu-HSC) permit *de novo* production of human T cells, B cells, monocytes/macrophages, and dendritic cells providing an ideal system to evaluate the species-jump by SIV. Here, we utilized hu-mice to simulate SIV serial transmission and repeated human exposures resulting in a human pathogenic virus. Hu-HSC mice were inoculated with the progenitor SIVcpz strains MB897 and LB715 (Group M), as well as EK505 (Group N). In contrast to previous studies which only analyzed single passage viral derivatives during short-term infection, we carried out multiple serial passages over two years duration using different cohorts of hu-mice. Viral adaptation was assessed by measuring plasma viral loads and $CD4^+$ T cell decline. Furthermore, genomic adaptation was evaluated using next-generation sequencing (NGS) by assessing the entire genomes of evolving viruses. All three SIV progenitors were able to infect hu-mice readily with viremia evident within 2 weeks which persisted through each generation. $CD4^+$ T cell decline was seen at varying levels amongst all three SIV strains during the first passage and the decline was more pronounced with each subsequent passage indicating increased pathogenicity. In contrast to previous studies wherein few mutations were noted in the *env* gene, we identified numerous nonsynonymous variants resulting in amino acid substitutions throughout the viral genome in *gag, pol, env* and *nef* regions, which increased in frequency by later passages/generations. These studies provided insight into how SIVs evolved towards HIV-1 following the initial cross-species transmission and subsequent spread amongst the human population.

6

Baboon Model of Innate Immunity against Immunodeficiency Virus Infection

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In its natural African non-human primate (NHP) hosts, SIV exposure generally results in nonpathogenic infection, despite active viral replication. Conversely, SIV infection in Asian rhesus macaques often progresses to AIDS. Circulating virus has never been recovered from baboons, an African NHP, despite thousands of years of direct exposure to SIV-infected NHP. Thus, baboons represent a potential model of innate immunodeficiency virus immunity. Our lab demonstrated SIV replication restriction in baboon PBMC compared to rhesus PBMC; however, infection in isolated baboon CD4 cells is equally permissive as in rhesus CD4. We adapted SIVmac by serial passage in baboon PBMC (SIVbn-PBMC) or isolated baboon CD4 cells (SIVbn-CD4) and analyzed changes to the genome by deep sequencing. Non-synonymous mutations appeared early in SIVbn-PBMC, whereas changes to SIVbn-CD4 were gradual and few became fixed, indicating that SIV faces stronger selective pressure in baboon PBMC versus isolated CD4 cells. We exposed two groups of naïve baboons to either SIVmac or SIVbn-PBMC. Surprisingly, after IV challenge, only SIVmac demonstrated active viral replication, which similarly to typical SIVmac infections in rhesus macaques, had viremia peaks at 2 weeks post-infection although at much lower viral loads. In baboons, SIVmac infection resulted in early peaks of CCR5-binding chemokine expression and blunted, transient type I interferon responses. RNAseq of sorted CD4 T, CD8 T and NK cells from baboons pre-infection and at peak viremia revealed an overall down regulation of genes associated with cell activation; CD4 T cells showed significant upregulation of BST-2. Anti-SIV antibody and cellular immune responses were detected in SIVmac-infected macaques, and virus could not be isolated from lymph nodes after 6 months-post-infection. In summary, infection of baboon with SIVmac was similar to the outcome seen in HIV elite controllers, whereas adaptation of SIVmac to baboon PBMC resulted in a virus unable to replicate in vivo.

7

SERINC5 Antagonism is Separable from other Nef Functions

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The Nef proteins of HIV-1 and SIV enhance viral infectivity by preventing the incorporation of the multipass transmembrane protein serine incorporator 5 (SERINC5), and to a lesser extent SERINC3, into virions. In addition to counteracting SERINCs, SIV Nef also downmodulates simian tetherin, CD4 and MHC class I (MHC I) molecules from the surface of infected cells. From a systematic analysis of alanine substitutions throughout the $SIV_{mac}239$ Nef protein, we identified two residues in the N-terminal domain that are specifically required to counteract SERINC5. Although there was significant overlap with sequences required for tetherin antagonism and CD4 downregulation, resistance to SERINC5 was genetically separable from other Nef functions. These results were used to engineer an infectious molecular clone of $SIV_{mac}239$ ($SIV_{mac}239$ Nef_{AV}), which differs by two amino acids in Nef that make the virus sensitive to SERINC5. This virus downmodulates CD3, CD4, MHC I and simian tetherin, but cannot counteract SERINC5. In primary rhesus macaque $CD4^+$ T cells, $SIV_{mac}239$ Nef_{AV} exhibits impaired infectivity and replication compared to wild-type $SIV_{mac}239$. These results demonstrate that SERINC5 antagonism can be separated from other Nef functions and reveal the impact of SERINC5 on lentiviral replication.

8

Th17-cells are early targets of SIV-infection during acute infection in Rhesus Macaque Model.

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Identification of infected cells immediately after mucosal HIV/SIV transmission is critical to design effective prevention strategies. Previous studies have identified CD4+ T-cells, especially, Th17s, as early targets of HIV/SIV in the female reproductive tract (FRT). Here we used rhesus macaque (RM) model to identify early targets of infection and temporal changes in target cell phenotypes. 12 female RMs were challenged intravaginally with a non-replicative luciferase reporter, LiCH, and SIVmac239 mixture. Animals were sacrificed either 48-, 72-, or 96-hours post-challenge. Macroscopic luciferase signal detected by *in vivo* imaging system (IVIS) allowed us to identify FRT regions likely containing infected cells. IVIS positive and negative tissues were serially cryosectioned for immunofluorescence staining and RNA isolation. Infected cells were phenotyped by microscopy to identify Th17s (CD3+CCR6+), other T-cells (CD3+), immature dendritic cells (iDCs)(CCR6+), and other cells (CD3-CCR6-). RNA was extracted from infected and non-infected adjacent tissue sections for RNA-Seq. Initial phenotypic analysis of SIV-infected cells in FRT of eight RMs sacrificed at 72hr and 96hr post-challenge identified infection throughout FRT in 3/4 and 4/4 of 72hr and 96hr animals, respectively, illustrating a vaginal squamous mucosa preference. Phenotypic profiling of 4,223 infected cells revealed that from 72 to 96hrs, proportion of Th17s remains constant (70-80%), however, an increase in infection rate of iDCs was observed (from 10 to 30%). Also, serial sectioning allowed us to observe the spread of infection in multiple sections of same tissue. These findings corroborate previous data identifying the entire FRT susceptible for infection with Th17s as early targets. Also, comparing the transcriptome profiles between infected and non-infected tissues at different time points will help to understand dynamics and kinetics of virus distribution during acute infection. Combined, these data will present a clearer picture of virus transmission at FRT mucosa and hence, aid in developing effective prevention approaches.

9

Identification and characterization of CD94 (KLRD1)+ memory-like NK cells in rhCMV- and SIV-infected rhesus macaques using RNA-flow cytometry

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Background: Recently others and we have shown that NK cells respond via memory-like recall responses during rhCMV/hCMV and SIV/HIV infection. These responses partly utilize the activating receptor NKG2C, and are associated with CD94 expression. Unfortunately, unfaithful antibody cross-reactivity renders it impossible to reliably detect CD94 or to distinguish NKG2C from its inhibitory counterpart, NKG2A, in nonhuman primates (NHP). Through our work we have shown that we can use NKG2A and NKG2C mRNA transcripts as reliable proxies for their protein expression via RNA-flow technology and here we apply this technology towards detecting CD94 in NHP. **Methods**: We phenotyped peripheral blood mononuclear cells from naïve and matched acute (n=4) or chronically (n=12) SIV-infected rhesus macaques using commercially available antibodies and determined the expression of transcripts for NKG2A, NKG2C and CD94 (KLRC1, KLRC2 and KLRD1 respectively) on NK cells using RNA-flow cytometry. **Results**: We detected KLRD1 expression on classically defined CD3-NKG2AC+ NK cells in both infected and uninfected cohorts, with no apparent differences in KLRD1 expression between the groups. Interestingly, our data also shows that the frequency of NKG2A+NKG2C+ NK cells is elevated by 30% in CD94+ NK cells following chronic SIV infection. We also show that upon co-culture with HLA-E high expressing K562 cells, NKG2A-NKG2C+ NK cells are unaffected whereas NKG2A+NKG2C+ NK cells exhibit a 24% reduction in cytotoxic potential as measured by CD107a levels. **Conclusions**: These results support the notion that the expression of NKG2A and NKG2C transcripts correspond to protein expression on the cell surface, and the substantial effect of HLA-E on NKG2A but not on NKG2C is in line with published literature. This approach will allow us to investigate the kinetics of infection and NK memory formation or exhaustion, and will further improve our understanding of basic NK cell biology, especially in the context of rhCMV or SIV infection.

10

The landscape of immune responses and persistent viral reservoirs in systemic and lymphoid tissues in rhesus macaques infected by a newly developed SHIV-CH848

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Background: The chimeric simian/human immunodeficiency viruses (SHIVs), carrying HIV envelope glycoproteins from transmitted founder (T/F) viruses, are valuable as a challenge viruses in nonhuman primate models for validating HIV-1 vaccines, assessing viral reservoirs and latency, and developing functional "cure" strategies in HIV infection. However, few SHIV strains have been shown to establish long-term and persistent viral infection and maintain viral reservoirs in tissues recapitulating human HIV infection. This study comprehensively investigated immunological changes, viral replication and reservoir size in deep tissues of SHIV-CH848-infected rhesus macaques on antiretroviral therapy (cART). **Material & Method:** A total of 10 rhesus macaques (*Macaca mulatta*, RMs) were intravenously (*i.v.)* inoculated with SHIV-CH848, containing *vpu-env* (gp140) sequence from T/F HIV-1 subtype C strain CH848. At 5 months post SHIV infection, 5 animals received cART (PMPA/FTC/DTG) for 6 months, and remaining 5 animals as untreated controls. Blood, lymph node and rectal biopsies were collected and plasma viral load, cell-associated viral RNA/DNA, and host immune responses were assessed. **Results:** Similar to HIV in humans and SIVmac in macaques, SHIV-CH848 inoculation resulted in rapid viral replication, reaching peak viremia at 14dpi and maintained detectable levels afterwards. SHIV-CH848 primary infection significantly depleted peripheral and mucosal CD4+ T cells, which represent the major viral reservoirs in both systemic and lymphoid tissues of SHIV-CH848-infected animals, compared with non-CD4+ T cells. Also, SHIV-CH848 infection induced PD-1 expressing CD8+ T cell dysfunction and exhaustion. Despite gradual recovery of CD4+ T cells after cART, the levels of integrated viral DNA in tissues were stable throughout 6-months of cART, and rapid viral rebound was observed after ART interruption. **Conclusions:** These findings suggest that the new SHIV strain, SHIV-CH848, is able to maintain persistent viral replication in macaques, and establish long-term viral reservoirs/latency in tissues, serving as a valuable model for HIV research.

Session II: Pathogenesis Pathogenesis of Primate Lentivirus Infections

Co-Chair: Dr. Michaela Muller-Trutwin

Tissue-specific viral control in a natural host of SIV

Since the discovery of HIV, tremendous progress has been made in preventing and treating HIV infection. However, a vaccine and a cure are still missing. Our laboratory aims to discover mechanisms that control HIV infection and HIV-associated disease. Our approach consists in learning from the efficient control mechanisms of HIV/AIDS that exist in some individuals infected by HIV and in rare animal models of spontaneous protection against AIDS, such as African green monkeys (AGM) infected by SIV. Natural hosts upon SIV infection display chronic high-level viremia but resolve inflammation and maintain an intact intestinal barrier. Several mechanisms have been also described on how natural hosts protect those cells and anatomical compartments which are key for the education and memory of immune responses, ie central memory CD4+ T cells and secondary lymphoid organs. We have previously uncovered an unprecedented mechanism of SIVagm control in secondary lymphoid organs of AGM mediated by NK cells. This control was associated with CXCR5 expression on NK cells and accumulation of NK cells in B cell follicles. We have further explored this NK-cell mediated control and recent results will be discussed.

11

Adipose tissue contributes to viral persistence in ART-treated SIV.sab infection in pigtailed macaques

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Background: Antiretroviral therapy (ART) effectively suppresses viremia in HIV-infected patients and SIV-infected RMs. Yet, ART does not restore immune integrity and is not curative, with the virus persisting in a latent reservoir and rebounding upon ART cessation. An increasing body of evidences suggests that adipose tissue (AdT) is a key anatomical reservoir that contributes to both viral persistence and chronic immune activation/inflammation. We used our new model of highly pathogenic SIV.sab infection of PTMs, treated with a coformulated combination of Emtricitabine [FTC], tenofovir disoproxil fumarate [PMPA] and Dolutegravir [DTG] to address the role of AdT during viral infection. **Methods:** Six SIVsab-infected PTMs received the coformulated regimen for 14 months from 48 days post-infection (dpi). Plasma viral loads (pVLs) were quantified by qRT-PCR assay. Cells isolated from the AdT were immunophenotyped by flow-cytometry and immunohistochemistry (IHC). DNAscope and cell-associated DNA (CA-DNA) were also performed to evaluate viral persistence in the AdT. **Results:**ART resulted in a robust viral control between 16 and 164 days post-treatment (dpt), with only rare blips occurring during the follow-up. Large numbers of T cells were observed in both white and brown AdT and they were located both perivascularly or diffuse in the fat. The majority of the CD4+ T cells isolated from the AdT was of central memory phenotype and expressed low levels of Ki-67 and HLA-DR suggesting low levels of activation. DNAscope showed virus persistence in the blood vessels cells from AdT. CA-DNA measurements in cells isolated from AdT and on snap-frozen tissue fragments of abdominal skin, peritoneal fat and pericardial fat collected at the necropsy confirmed AdT as a virus reservoir. By IHC we showed that the cells infiltrating the AdT produce IL-6 and have an increased expression of MXA-1, indicating AdT as a source of residual INFL. **Conclusion:**Our study shows that the AdT is a major anatomical site of virus persistence and immune activation/inflammation in a new model of ART-treated SIV infection in Pigtail macaques. These findings confirm that AdT should be targeted by the HIV/SIV cure strategies.

12

Rapid progression in SIV-infected infant rhesus macaques is associated with failure to increase CXCL13 during acute infection, B cell dysfunction and early viral replication

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Immunological factors influencing rapid progression to AIDS observed in infants following vertical HIV transmission remain poorly understood. In particular it is not known why some infants mount anti-HIV humoral responses, typically associated with chronic infection, while others fail to do so and progress rapidly to AIDS. To address this question, 25 infant rhesus macaques were orally infected with SIVmac251 and monitored for 10 to 17 weeks post-infection. Infants were divided into rapid (RP) or typical (TypP) progressors based on viral loads and plasma anti-SIV antibody levels. Infants exhibiting the RP phenotype had higher chronic viral loads, low/undetectable levels of SIV-specific antibodies and were more likely to develop AIDS-related illnesses. Our findings indicated that the RP phenotype was associated with B cell dysfunction, and not CD4 levels nor generalized immune activation. Indeed, infants in the RP group had 9.8 fold fewer activated memory B cells in blood during chronic infection and significantly lower proportions of germinal center (GC) B cells and Tfh cells as well as increased activated plasmacytoid dendritic cells in axillary lymph nodes sampled at 4- and 10-weeks post-SIV. Image analysis revealed significantly fewer proliferating splenic germinal centers associated with elevated levels of SIV+ cells and type-1 interferon protein expression (MX1) in RP infants. Interestingly, at 2-weeks post-SIV, RP infants had significantly higher numbers of unique envelope variants (mean 28 in TypP vs 46 in RP) while failing to increase plasma concentrations of the GC-inducing chemokine CXCL13. Our findings suggest that differences in acute viral replication and increased type-1 interferon during pre-chronic infection impedes the development of anti-SIV antibody responses. We hypothesize that early dysfunction in germinal center formation may contribute to the rapid AIDS progression observed in human infants.

13

Modulation of host responses to HIV infection by long non-coding RNAs

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HIV-1 replication requires the functions of hundreds of protein-coding host factors as revealed by several genome-wide RNA interference (RNAi)-based screening and CRISPR screening studies. These screens, however, did not include long non-coding RNAs (lncRNA) genes, which constitute 70% of the cellular transcriptome and regulate immune cell development, differentiation and response to infections, including that of human CD4⁺ T cells and macrophages. LncRNAs can significantly affect the expression and functions of protein-coding genes. The pervasive influence of lncRNAs on gene regulation makes them an attractive target for viruses to hijack the cellular gene machinery and enhance their propagation. Though thousands of lncRNA transcripts are expressed HIV target CD4⁺ T cells and myeloid cells, their functional impact on HIV replication and immune responses has been determined for only a few lncRNAs. Currently, the effect of cellular lncRNAs in HIV pathogenesis and the underlying molecular mechanisms are unclear. Using computational and experimental approaches, we observed significant global changes in cellular lncRNA expression patterns in HIV-infected CD4⁺T cells. Bioinformatics analyses indicated a significant correlation between the expression of the lncRNAs and protein-coding transcripts, which play an important role in anti-viral immunity. Several HIV-induced lncRNAs are expressed show significant differential expression among elite controllers as compared to chronic HIV patients or uninfected donors. Our studies indicate a novel role of lncRNAs in significantly modulating host responses to HIV infection.

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Chronic morphine administration affects SIV reservoirs

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The Opioid crisis" is a national drug epidemic in USA, that has also significantly impacted people living with HIV-1. Opioids modulate immune responses and negatively impact adherence to combined antiretroviral therapy (cART). HIV reservoirs persist despite effective cART suppression on peripheral viral loads. The effect of opioids on viral reservoir dynamics, however, remains elusive. Herein, we sought to examine the impact of morphine dependency on the size of SIV reservoirs using the rhesus macaque (RM) model. Eleven RMs (six morphine-dependent (daily) and five saline controls) were dosed for 8 weeks and infected intravenously with SIVmac251. cART was initiated 5 weeks post-infection and plasma/CSF viral loads and immunophenotyping of PBMCs were assessed longitudinally. Cell-associated DNA was quantified using ddPCR on PBMC/LN/Gut biopsies. Next, we also assessed the size of inducible viral reservoir using the Tat/Rev Induced Limited Dilution Assay (TILDA) and T-cell polarization by intracellular cytokine staining. There were no significant differences in the plasma/CSF viral loads or CD4⁺/CD8⁺ T cell counts between the two groups. However, we observed the median of total cell associated DNA loads were lower within peripheral blood CD4⁺ T cells (no statistical significance) and rectal biopsy (p=0.0303); Next, the size of inducible viral reservoirs in peripheral blood/LN was reduced in the morphine group as compared to the saline group (no statistical significance). Finally, the intracellular cytokine staining on CD4⁺ T cell subsets showed a decreased frequency of circulating Th1 and Th17 CD4⁺ T cells with an increase in Treg cells in the morphine-dependent group compared to controls. These findings thus suggest that morphine is likely involved in reducing HIV/SIV reservoirs both in the peripheral blood and tissues. Future studies using a larger cohort of animals with therapeutic relevant doses of morphine are warranted, to make definitive conclusions on the impact of morphine on HIV/SIV reservoirs.

Session II: Pathogenesis Pathogenesis of Primate Lentivirus Infections

Co-Chair: Dr. David Evans

Resistance of HIV- and SIV-infected cells to elimination by antibodies

HIV-1 and SIV have evolved to replicate continuously in the presence of ongoing host immune responses and have therefore acquired mechanisms to evade all immune responses, including Fc-mediated functions of antibodies such as antibody-dependent cellular cytotoxicity (ADCC). We and others have identified molecular mechanisms that contribute to the resistance of HIV-infected cells to ADCC. I will review these mechanisms and present data from recent passive transfer experiments with an antibody that uncouples ADCC from virus neutralization.

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Monocyte turnover increase reflects tissue macrophage destruction and disease pro-gression to AIDS in SIV-infected rhesus macaques.

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Background: Declining CD4+ T cells is considered a hallmark of HIV/AIDS pathogenesis. We recently demonstrated that tissue macrophage destruction by SIV as reflected by increased monocyte turnover (MTO) in blood also factors into AIDS pathogenesis and may better predict onset of disease progression. To further explore this, we examined MTO levels over time in macaques infected with SIV strains including the "pathogenic" SHIV that massively depletes CD4+ T cells soon after infection. **Methods**: Rhesus macaques were infected with SIVmac251/239, SIV0302, SIVmac316e, or SHIV89.6P. MTO was monitored 24 hours after each BrdU injection by immunostaining and flow cytometry of blood. **Results:** Regardless of the SIV strain (macrophage- or T cell-tropic), infected macaques exhibited MTO increases over baseline just prior to disease progression independent of time post infection. Conversely, CD4+ T cell levels gradually declined but less consistently to onset of disease progression. SHIV89.6P virus-infected macaques, for example, all exhibited dramatically-reduced CD4+ T cells following infection, but two of the animals survived until study endpoint (~8 months post infection) without overt clinical signs of disease and without MTO increase. The two other animals that required euthanasia 4.5 months post infection did exhibit clinical signs of disease and increased MTO. There also was a significant inverse correlation between MTO levels during the chronic phase and time until death among all animals examined here ($r = -0.777$; $P < 0.0001$). **Discussion:** These results further support that increasing MTO levels more consistently predict disease progression of AIDS compared to declining CD4+ T cells. The increase in MTO also appears to reflect tissue macrophage destruction by SIV which likely is critical to tissue damage and onset of terminal AIDS disease progression. Overall, this work continues to support a critical role by monocytes/macrophages in attempting to maintain homeostasis despite massive tissue macrophage destruction by SIV.

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Session II: Pathogenesis Pathogenesis of Primate Lentivirus Infections

Co-Chair: Dr. Mahesh Mohan

Long term delta-9-tetrahydrocannabinol administration inhibits oral mucosal inflammation in chronically SIV-infected rhesus macaques through regulating microRNA mediated pathways and the oral microbiome

HIV/SIV associated oral mucosal disease/dysfunction (HIOD) (gingivitis/periodontitis/salivary adenitis) represents a major comorbidity affecting patients on anti-retroviral therapy. A systems biology approach was used to investigate the molecular changes and potential mechanisms underlying HIV/SIV associated oral mucosal dysfunction/disease. Specifically, using RNA-seq and TaqMan OpenArray panels we identified significant changes in proinflammatory gene and microRNA expression, respectively in the oral mucosa of vehicle treated SIV-infected macaques. Interestingly, chronic delta-9-tetrahydrocannabinol treatment significantly attenuated these changes. We characterized three important proteins associated with endoplasmic reticulum stress (AGR2), epithelial barrier maintenance and anti-dysbiosis (WFDC2) and glucocorticoid and IL10 induced anti-inflammatory TSC22D3 (also known as GILZ). Immunofluorescence studies identified the acini and secretory duct of minor salivary glands in the oral mucosa as the major cellular source of these proteins and confirmed enhanced expression in oral mucosa of THC treated SIV-infected macaques. Finally, THC treatment prevented oral dysbiosis by preserving the abundance of *Lactobacilli* and inhibiting the expansion of *Streptococci* and periodontitis causing *Prevotella* species.

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Functional perturbation of mucosal group 3 innate lymphoid and natural killer cells in SHIV.CH505-infected neonatal rhesus macaques

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Background: Mother-to-child transmission of HIV-1 via breastfeeding is responsible for roughly half of children newly infected with HIV. Although ILC3 and NKs are also found throughout the oral mucosae, the effects of HIV/SHIV in these tissues are largely unknown. To better understand the mechanics of postnatal transmission we performed a comprehensive study of SHIV-infected neonate rhesus macaques and tracked changes in frequency, trafficking, and function of aforementioned cells as a result of acute infection. Materials/Methods: We evaluated ILC3s and NKs in cohorts of naïve and lentivirus-infected neonatal rhesus macaques. ILC3s and NKs were identified using lineage markers (CD45+/CD3-/CD14-NKG2a/c-/NKp44+, CD45+/CD3-/NKG2a/c+/NKp44- , respectively). Additional phenotypic measures were conducted by mitogen stimulation and traditional flow-cytometry and analyzed using SPICE. Results: Infection led to a 3-fold depletion of ILC3s in the colon ($p = 0.0011$) and an increase in NKs in tonsils and Sub LN ($p = 0.0439$, 0.066 respectively). ILC3s and NKs saw alterations in their trafficking repertoires as a result of SHIVCH505-infection, with increased expression of CD103 in colon NKs and curtailment of CXCR3. Meanwhile, infection induced an increase in CD62L and CXCR3 and significant decrease in a4b7 expression in colon ILC3s. SPICE revealed that ILC3s and NKs had distinct functional profiles by tissue in naïve samples. ILC3s and NKs from the Sub LNs displayed a significantly different functional repertoire than those from the colon and NKs from the colon also with those from the tonsil. Infection perturbed these profiles, with a near total loss of IL-22 production in the tonsil and colon and increase in CD107a, IFNy and TNFa from ILC3s, and increase in CD107a, MIP-1b and TNFa from NKs. Conclusion: Collectively, these data reveal that acute SHIV-infection alters the frequency, trafficking and cytokine receptors of innate cells in the oral and gut mucosa, along with the functional profiles of said cells.

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Enhanced Protection at the Site of Challenge of Rhesus Macaques That Receive PGT121 One Week Prior to Intravaginal Challenge With SHIV-SF162P3

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Background: In a recent study, rhesus macaques(RM) that got an intravenous(IV) infusion of PGT121, 24hrs prior to intravaginal challenge with SHIV-SF162P3, had distal site accumulation of virus 1-3 days after challenge. Using Cy5-labeled VRC01 IV-injected into RMs we found that it takes antibodies ~1wk to achieve steady-state tissue levels. The aim of this study is to determine if giving antibodies more time to fully distribute can block distal site accumulation of virus following intravaginal challenge. **Methods:** Utilizing Cy5-PGT121 and sham antibody Cy5-DEN3, we compared -1wk(n=5) and -24hrs(n=5) IV infusion prior to intravaginal challenge with SHIV-SF162P3 in RM and measured virus 48hrs after challenge. Tissue and plasma levels of viral RNA and DNA were detected using gag qPCR and antibody levels were measured through Cy5 fluorescence using deconvolution microscopy and a fluorometer. **Results:** Whereas we detected viral DNA at the site of challenge in all the DEN3 and -1 day PGT121 RMs, we only detected viral DNA in 1/5 RMs in the -7 day PGT121 group. In a small subset of animals(2/5) in both PGT121 groups we detected viral DNA in lymph nodes(LN), with more sites in the -7 day group. In these distal site tissues where we observed viral DNA, PGT121 was also present. Importantly in these animals that had increased viral DNA in the LN, there was no viral RNA detected in the blood. RMs that received DEN3 had no distal site accumulation of viral DNA. **Conclusions:** We have found that despite giving antibodies more time to achieve steady state at the site of challenge, there was still distal site accumulation of viral DNA 48 hours after challenge in a small subset of animals. Since this does not occur in DEN3-injected RM, the early distal site accumulation of viral DNA in the LN appears to be PGT121 dependent.

Session III: Co-infections in the NHP Model

Co-Chair: Dr. Marcelo Kuroda

Macrophage damage by SIV is critical for TB reactivation and AIDS disease progression in rhesus macaques

Mycobacterium tuberculosis (Mtb) and HIV interact synergistically to accelerate disease progression, but the mechanisms underlying pathogenesis of Mtb/HIV co-infection are not fully understood. Immunodeficiency from CD4+ T-cell depletion by HIV infection is believed to be a major factor contributing to opportunistic infections including tuberculosis (TB) reactivation. Results from our studies using the rhesus macaque SIV and Mtb co-infection model, however, demonstrated that tissue macrophage damage/death by SIV infection preceded and predicted the onset of AIDS disease progression as well as reactivation of latent TB infection better than declining CD4+ T cells. These data demonstrate a critical role of short-lived macrophage destruction as a mechanism of AIDS disease pathogenesis and TB reactivation while long-lived macrophages may contribute to the virus reservoir and chronic inflammation.

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Chronic immune activation due to SIV co-infection correlates with reactivation of latent tuberculosis infection

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HIV is a major driver of Tuberculosis (TB) reactivation. Depletion of $CD4^+$ T cells is assumed to be the basis behind TB reactivation in individuals with latent tuberculosis Infection (LTBI) co-infected with human immunodeficiency virus (HIV). Here, we show that non-human primates (NHPs) coinfected with a mutant simian immunodeficiency virus (SIVΔGY),that does not cause depletion of tissue $CD4^+$T cells during infection, failed to reactivate TB. To investigate the contribution of $CD4^+$T cell depletion relative to other mechanisms of SIV-induced reactivation of LTBI, we used CD4R1 antibody to deplete $CD4^+$ T cells in animals with LTBI without lentiviral infection. We show that the mere depletion of $CD4^+$ T cells during LTBI is insufficient to cause reactivation of LTBI. Instead, direct cytopathic effects of SIV resulting in chronic immune activation, along with the altered effector T cell phenotypes and dysregulated T cell homeostasis, are critical mediators of reactivation of LTBI. These results reveal important implications for controlling TB in the HIV co-infected individuals.

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A Non-human Primate Model for Vaginally Acquired Syphilis

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Background: With increasing numbers of syphilis cases domestically, non-human primate (NHP) models may facilitate evaluation of HIV biomedical interventions in the context of sexually transmitted infections (STIs). We previously developed a vaginal model of *Chlamydia* and *Trichomonas* co-infections, and the first macaque model for rectally acquired syphilis. Here, we present a vaginal syphilis model involving co-infection with one or more STIs. **Methods:** Six naïve pigtailed macaques were first infected with *Chlamydia trachomatis* (CT, $1x10^6$ IFU), and *Trichomonas vaginalis* (TV, $6x10^6$ trichomonads), followed by exposure to 10^8 organisms/mL *Treponema pallidum* (TP) Nichols strain (200µL; 4 submucosal injections) in the cervicovaginal site. Six additional SHIV-infected macaques were exposed to TP; four were co-infected with CT and TV. All six SHIV-infected animals were re-exposed to TP after their initial lesions had healed. The lesions were monitored by colposcopy using narrow band imaging. Syphilis testing involved serological evaluation by treponemal (*Treponema pallidum* particle agglutination assay, TP-PA; Trep-Sure™) and non-treponemal (rapid plasma reagin, RPR) tests. CT and TV infections were confirmed by Hologic Aptima Combo® 2 assay. **Results:** All 12 animals showed cervicovaginal lesions at a median of 11 days (range 7-18 days). In the SHIV-positive animals re-exposed to TP, lesions re-appeared within a median of 14 days (range 7-18 days). Vaginal lesions resolved later than rectal lesions (median, 76 days versus 20 days, respectively). Seroconversion in the six SHIV-infected macaques occurred at a median of 37.5 days (range 7-53 days), with a peak TP-PA titer of 1:20,480 at a median of 96 days, after which titers plateaued. The six SHIV-naïve macaques have seroconverted and are being monitored. **Conclusions:** We have successfully developed the first NHP model establishing vaginal syphilis acquisition in the context of epidemiologically relevant STIs. We have demonstrated seroconversion, mucosal lesions, and re-infection with TP.

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Characterization of MAIT cells in Mauritian cynomolgus macaques (MCM) SIV/Mtb co-infection

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The impact of HIV-infection on the contribution of Mucosal Associated Invariant T (MAIT) cells to the control of M. tuberculosis (Mtb) infection is unknown. We developed a nonhuman primate model of SIV/Mtb co-infection to better understand the mechanism by which SIV, as a model for HIV, weakens host immune responses to Mtb. In this study, we tested the hypothesis that a pre-existing SIV infection dysregulates MAIT cell frequency and function upon co-infection with Mtb. To test this hypothesis, we infected SIV+ and SIV- naïve macaques with a low dose of Mtb Erdman (~10 CFU). Animals were necropsied at 6 weeks post Mtb infection to characterize MAIT cell frequency and function in the individual tissues. We necropsied an additional cohort of animals 6 months after SIV infection, at the time the other cohort was co-infected with Mtb in order to characterize MAIT cells in the tissues just prior to co-infection. Across all groups of animals, we found no marked changes in the frequency and function of MAIT cells from SIV+, SIV+/Mtb+ and SIV-/Mtb+ macaques. Notably, we found that MAIT cells from SIV+ and SIV+/Mtb+ animals exhibited higher expression of PD-1 and TIGIT in the tissues, although this did not appear to affect their function when cells were subjected to in vitro stimulation assays with fixed bacteria. Overall, based on the assays used here to test the function of MAIT cells, these data suggest that dysregulation of MAIT cells by SIV infection may not affect directly anti-mycobacterial MAIT cell function. Future studies will be needed to reveal if alternative unconventional T cells play a greater role.

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Rhesus Macaques Support Chronic HBV infection in vivo and are an emerging model of HIV/HBV co-infection

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HIV and HBV are both major global health concerns. HIV infects 37 million people while HBV infects 257 million individuals worldwide, both causing around a million deaths annually. HIV-infected patients that subsequently acquire HBV progress to chronic HBV infection more frequently than HIV-naïve individuals, and HIV/HBV co-infected individuals have higher HBV viral loads, reduced HBV-specific T cell responses, and a higher probability of extensive liver fibrosis and hepatocellular carcinomas. Thus, a greater understanding of the interplay between HIV and HBV infections is urgently needed to design strategies to prevent accelerated liver disease. Chimpanzees have long been the gold standard for primate HBV research, but all chimpanzee research is now under moratorium. Indeed, one of the major obstacles to the understanding of HIV/HBV co-infections is the lack of a physiologically-relevant primate model. We previously published that rhesus macaque (RM) hepatocytes transduced with viral vectors expressing the HBV receptor, human sodium taurocholate co-transporting polypeptide (hNTCP), support HBV infection both *in vitro* and *in vivo*. However, we observed only low-level, transient HBV viremia *in vivo*. Here, we present a vastly improved RM chronic HBV infection model with robust HBV replication defined by persistently high levels of HBV plasma viremia (>10e6 copies/ml) accompanied by high levels of HBV surface and envelope antigens in blood for more than six months. In addition, high frequencies of HBV core and surface antigen-positive hepatocytes are present longitudinally in the liver. Taken together, our data indicate that RM represent a promising emerging model to study HIV/HBV co-infections and that additional research should be dedicated to the model's further development.

Session III: Co-infections in the NHP Model

Co-Chair: Dr. Deepak Kaushal

HIV and TB: New Approaches to Treating the Syndemic

HIV is the most critical risk factor for reactivation of latent tuberculosis infection (LTBI) in highly endemic regions of TB. While CD4+ T cell depletion has been considered the major cause of LTBI reactivation, our recent work in macaques co-infected with Mtb/SIV suggests that the extensive reactivation of LTBI is in fact, due to indirect cytopathic effects of SIV resulting in chronic immune activation and a dysregulation in T cell homeostasis. This presentation builds on compelling data that the reactivation of LTBI during HIV co-infection is likely driven by the events of HIV replication and therefore highlights the need to have optimum translational interventions directed at reactivation due to co-infection.

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Fetal brain transcriptomic signature of congenital Zika syndrome identifies alterations in the myelination process

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Background: Zika virus (ZIKV) infection during pregnancy causes a wide array of fetal abnormalities, including cerebral atrophy and intrauterine growth restriction. At present, there is no available vaccine or therapeutic to prevent ZIKV vertical transmission. NHP models of ZIKV infection in pregnancy have demonstrated vertical transmission and fetal neuropathology; however, the host response to ZIKV infection in the fetal brain remains to be elucidated. Here, we performed a transcriptomic analysis of pigtail macaque fetal brain to characterize host transcriptional responses in fetuses exposed to ZIKV *in utero*. Materials & Methods: Five, healthy pregnant pigtail macaques received subcutaneous inoculations of ZIKV in the forearms. At birth, the fetal brain was serially sectioned to collect samples for histologic, TEM and RNA analyses. For each animal, we performed Total RNA-seq on five individual brain samples containing white matter and spanning frontal (R1 and R3), parietal (R5 and R7) and occipital (R9) brain regions. Results: We found increased expression of innate immune and inflammatory pathway genes in the occipital region containing the lesion. EM analysis of the posterior brain showed severe tissue destruction, concomitant with the transcriptomics and MRI finding of T2-hyperintense foci surrounding the lateral ventricle. In the parietal region, we identified decreased expression of genes related to myelination. Immunohistochemical analysis showed decreased expression of myelin basic protein (MBP) in ZIKV-infected fetal brain compared to control brains. Gene co-expression network analysis further identified myelination-associated genes altered in response to ZIKV infection. Conclusions: Our data sets and computational approaches define fetal transcriptional profiles linked with ZIKV-induced phenotypic outcomes. Decreased MBP expression and myelin-associated gene expression in the fetal brain is the result of ZIKV infection during pregnancy. Oligodendrocytes express MBP that form the myelin sheath surrounding axons and this process may be impacted in infant neurodevelopment as a consequence of ZIKV exposure *in utero*.

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Early induction of Mycobacterial lipid antigen-specific responses in BCG-inoculated macaques and impact of SIV coinfection on these responses

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Tuberculosis (TB) vaccine strategies have historically focused on conventional T cell responses to peptide antigens. However, there is growing evidence for the potential role of unconventional T cell responses to lipid antigens in TB immunity. Importantly, lipid-reactive T cells may play a crucial role during HIV coinfection, which significantly increases the risk of latent TB reactivation by impairment of conventional T cell responses. In this study, we utilized six cynomolgus macaques (CM) to characterize mycobacterial lipid antigen-specific T cell responses following aerosol BCG exposure, and subsequent SIV-coinfection. Responses to total cellular lipids of *M.bovis* or *MTb* H37Rv, or purified H37Rv lipid antigens, including Lipoarabinomannan (LAM), Phosphatidylinositol mannoside (PIM-6) and mycolic acid were examined *ex vivo* in peripheral blood and bronchioalveolar lavage (BAL) mononuclear cells by IFN-γ and GranzymeB ELISPOT assays and Flow cytometry. Innate lipid antigen-specific responses were observed in naïve CM that were further boosted following aerosol exposure to BCG. Post-exposure lipid-specific responses preceded peptide responses and were comparable in magnitude to TB10.4 antigen. Besides a dominant Th1 effector function, lipid-reactive T cells displayed CD4-negative phenotype, suggesting that they are not direct targets of SIV. However, the effector responses in BAL were distinct from PBMC both in quality and magnitude. Acute SIV infection significantly impacted cytokine responses in blood in contrast to cytotoxic functions in BAL, suggesting differential modulation of immune responses in blood and lungs. These results suggest that the robust Th1-type/cytotoxic response of lipid-reactive T cells following mycobacterial exposure are a significant component of the early immune response to mycobacterial infection, during which adaptive immunity develops and SIV infection impacts these responses. Thus, understanding lipid-reactive T cell functions in response to mycobacterial exposure, particularly in lungs, may provide critical insights into novel mechanisms of resistance to TB and aid the development of new therapies.

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Impact of P. fragile infection on SHIV.CH505 acquisition and viral load in rhesus macaques

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Background: Given the substantial overlap in the geographical areas in which HIV and malaria are endemic, the potential for co-infection is considerable and could fuel the spread of both diseases. However, the influence of malaria infection on risk of mucosal acquisition of HIV infection is not well understood. Here, we assessed the impact of *Plasmodium fragile* infection on SHIV acquisition and post-infection viral kinetics in rhesus macaques. **Methods:** Male rhesus macaques (n=8) were intravenously inoculated with *P. fragile.* Productive *P. fragile* infection was confirmed by Giemsa staining of thin blood smears. *P. fragile*-uninfected male rhesus macaques (n=11) were used as controls. All animals underwent multiple low-dose intra-rectal challenges with SHIV.CH505. Viral challenge was halted when animals tested PCR positive for viral sequences in plasma. **Results:** All animals became productively infected with SHIV.CH505 within 9 challenges. No significant differences were observed in the rate of SHIV acquisition between *P. fragile*-infected and uninfected animals. In all animals, peak viral load was reached by week2 post-SHIV infection. Median peak viral load ($1.01x10^6$ [$1.1x10^5$ – $1.57x10^7$] RNA copies/ml plasma) and median set point viral load ($7.6x10^3$ [19 – $2.6x10^4$] RNA copies/ml plasma) was higher in *P. fragile*-infected animals as compared to *P. fragile*-uninfected animals ($3.5x10^5$ [$2.9x10^4$ – $5.5x10^6$] and $7.6x10^3$ [19 – $2.6x10^4$] RNA copies/ml plasma, respectively), although these differences did not reach statistical significance. **Conclusions:** Our initial investigations in this ongoing study indicate that *P. fragile* infection does not influence the rate of SHIV.CH505 acquisition, peak or set point viral load in male rhesus macaques. Additional animals are currently being assessed, which will increase the power of this study. Furthermore, we are characterizing the impact of *P. fragile* infection on mucosal immune subsets prior to and throughout SHIV infection. Our work will provide a comprehensive assessment of the effect of malaria co-infection on SHIV acquisition and pathogenesis.

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Maintenance of Mtb antigen specific CXCR3 and CCR6 co-expressing cells in BAL are associated with immune control in LTBI Rhesus Macaques

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Immune responses associated with immune control of LTBI are not known and the CD4 and CD8 T cell responses in blood and lung compartments are poorly understood. Here, using a non-human primate (NHP) model for latent TB (LTBI), we studied the kinetics and phenotype of Mtb-specific T cells at the site of infection that are involved in immune control following aerosol infection. Rhesus macaques (6 animals) were infected through aerosol route with low dose Mtb strain CDC 1551. Infection was assessed using Tuberculin Skin test, and Interferon Gamma Release Assays and latency was determined by chest x-ray and lack of clinical signs and symptoms. Animals were longitudinally followed until week 24. We studied the phenotype and function of Mtb specific T cells in peripheral blood, BAL by flow cytometry. We observed Mtb-specific responses at week 3 in BAL which peaked at week 7 and its frequency were maintained throughout the latent infection. Mtb-antigen specific T cells producing IFN-γ and IL-17 were significantly higher in BAL compared to PBMC. The frequency of Mtb-specific activated T cells expressing CD38 in BAL observed at week 3 were maintained until week 24 (necropsy) indicating immune control. Mtb-antigen specific T cells were of central memory phenotype and expressed both CCR6 and CXCR3. Immunohistochemical staining of lung tissues at necropsy revealed that the majority of CD4+ T cells were expressing CXCR3 and were located in the non-granulomatous area compared to the granuloma area of the lung. Thus, using the NHP latency model, we show that Mtb-specific T cells involved in immune control at the local site are maintained throughout latent infection that display memory phenotype and express both CXCR3 and CCR6. Persistence of Mtb antigen specific T cells at the site of infection might be associated immune control of Mtb, which highlights the importance of vaccine and therapeutic strategies to elicit these response in vivo.

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Can cART alone deliver in the race between immune reconstitution and TB reactivation?

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Background: TB and HIV syndemic has resulted in higher incidence of drug resistant TB leading to poor treatment outcomes and increased mortality. The advent of combined antiretroviral therapy (*cART*) has significantly improved survival but TB still accounts for one third of deaths in HIV infected population, suggesting incomplete restoration of immune function by *cART*. Design: To identify the components of immunity that remain impaired following cART therapy in Mtb/SIV co-infection, twelve Indian-origin rhesus macaques (*Macaca mulatta*) were infected with aerosolized low dose ~10 CFU of *Mtb* CDC1551. Animals were studied longitudinally for their clinical and immunological responses and upon confirming their latent TB status, they were challenged with intravenous simian immunodeficiency virus $SIVmac_{239}$ 300 $TCID_{50}$ at week 9 post *Mtb* infection. At week 13, animals were randomized to control group and treatment group and initiated on *cART* consisting of tenofovir, emtricitabine and dolutegravir administered subcutaneously. Results: *cART* significantly reduced plasma viral load and increased CD4+ T cell counts in whole blood and bronchoalveolar lavage samples. Despite reduced viral load and improved CD4+ T cell count, however, there was no change in SIV-induced TB reactivation. Increased Th1 responses were observed in restored CD4+ T cells, indicating TB-IRIS like phenomena. Conclusion: We conclude that early *cART* therapy is indispensable for controlling viral replication, CD4+ T cells restoration and in preventing opportunistic infections. Albeit the beneficial effects of *cART* inversely correlate with delay in onset of treatment, *Mtb* antigen load, CD4+ T cell depletion and level of Th1 immune activation. With IRIS being an early fatal complication of *cART* especially in *Mtb/HIV* co-infection cases, it is pertinent to control the *Mtb* antigenic load with adjuvant prophylactic anti-tuberculosis treatment before or in concurrence with initiation of *cART*.

Session IV: Therapeutics/Cure in the NHP Model

Co-Chair: Dr. Susana Valente

Silencing the HIV-1 reservoir, The "block and Lock" approach

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Didehydro-Cortistatin A (dCA), is a very potent inhibitor of HIV-1 transcription (Mousseau et al. Cell Host and Microbe, 2012). dCA binds to the basic domain of the viral Tat protein and inhibits Tat activated transcription at sub-nanomolar concentrations (Mediouni et al, mBio, 2019). As such, dCA stops the Tat feedback-loop and overtime prompts the viral promoter into deep transcriptional inhibition, resistant to viral reactivation (Mousseau et al. mBio, 2015; Li et al. Epigenetics and Chromatin, 2019). Based on the mode of action of dCA, we proposed a functional HIV cure strategy, dubbed "block-and-lock". The premise is that the combination of ART with an HIV transcriptional inhibitor, such as dCA, would "block" ongoing viral replication, resulting in the accumulation of epigenetic marks at the latent loci that "lock" HIV transcription in a latent state. We have shown in human CD4+T cells isolated from aviremic individuals that combining dCA with ART accelerates HIV-1 suppression and prevents viral rebound during treatment interruption. In the bone marrow-liver-thymus (BLT) mouse model of HIV latency and persistence, adding dCA to ART suppressed mice, reduces viral RNA in tissues, and significantly delays and diminishes viral rebound upon treatment interruption (Kessing et al, Cell Reports, 2017). The "block-and-lock" approach is now widely accepted, and we are proud to have proved the concept. To date, dCA is the most promising Tat inhibitor with the ability to transform the way we treat HIV-1 infections.

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CD4-MBL-CAR/CXCR5 T cells suppress SIV replication post-ART release

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HIV and SIV replication is concentrated within lymphoid follicles during chronic infection, where low levels of virus-specific CTL permit ongoing viral replication. Elevated levels of SIV-specific CD8 T cells in B cell follicles are linked to decreased levels of viral replication in follicles and decreased plasma viral loads. These findings provide the rationale for the development of a strategy for targeting follicular viral replication using T cells co-expressing an antiviral chimeric antigen receptor (CAR), specifically a rhesus CD4-MBL-CAR, and the follicular homing chemokine receptor CXCR5. We hypothesize that CAR/CXCR5 T cells can target virally infected cells in follicles, and lead to sustained remission of SIV/HIV. To test this hypothesis, we engineered CAR/CXCR5 T cells and used them in studies in which CAR/CXCR5 cells were labeled with the live cell stain CTV and infused into rhesus macaques. In our initial study, CAR/CXCR5 cells were infused into an SIV-infected rhesus macaque and tissues were evaluated 2 days later. Next, we evaluated six SIV-infected ART-suppressed rhesus macaques in which CAR/CXCR5 cells were infused and animals were released from ART. Three untreated animals were included as controls. CAR/CXCr5 cells were detected proliferating in extrafollicular areas and within follicles of lymphoid tissues at 2 days post-infusion. Levels of CAR/CXCR5 T cells increased in follicles at 6 days post-infusion. CAR/CXCR5 T cells were found in contact with vRNA+ cells in follicles. All treated animals showed viral suppression early after ART release. One month post-infusion viral loads were substantially reduced in treated compared to control animals. Increased doses of CAR/CXCR5 T cells were associated with significant reductions in viral loads. These studies show that CD4-MBL-CAR/CXCR5 immunotherapy is safe and effective.

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Distribution of I.V. injected Cu64, Zr89, and fluorescently labeled VRC01 and VRC01-LS in the in vivo Rhesus Macaque Model

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Antibody-mediated protection has been illustrated in non-human primates; however, it's still unknown how antibodies are anatomically distributed. Using the living rhesus macaque (RM) model, correlative positron emission tomography (PET), light-sheet microscopy (LSM), and deconvolution microscopy (DVM), we are able to observe unique antibody distributions amongst organs and within tissues. These studies have allowed us to elucidate antibody distribution over time, providing insights into the mechanism(s) of antibody delivery to those mucosal tissues important in HIV transmission. For PET studies, RMs were IV-administered Cu64- or Zr89-tagged VRC01 or VRC01-LS and scanned for up to 72hours or 3weeks, respectively. To assess antibody distribution with LSM and DVM, 14 RMs were IV-administered fluorescently tagged VRC01 and/or VRC01-LS, with 4 macaques DMPA-treated ~30days prior to administration. Following, necropsy tissues were collected. For DVM, tissues were stained with an anti-FcRn. With PET, we visualize differences in VRC01 and VRC01-LS distribution between whole organs, across multiple time points. Using microscopy, we identify differences of antibody distribution amongst various mucosal tissues: In squamous tissues, we see antibodies being delivered with epithelial differentiation. In columnar and brain tissues we visualize antibody distribution through the vascular system, with antibody-FcRn interactions. We also show that VRC01-LS is able to persist longer in tissues than VRC01 due to endosomal recycling within endothelial cells. Lastly, we observe antibody differences in DMPA-treated animals, but with studies still ongoing. Here, our data illustrate a variety of methodologies for antibody delivery to anatomical sites. Using correlative PET, we find that whole organ antibody distribution varies between VRC01 and VRC01-LS. Using LSM and DVM with anti-FcRn antibodies, we are able to further understand the intricacies of these distribution differences, including how DMPA may affect *in vivo* antibody distribution. These studies start to unravel the complexities of antibody delivery to sites involved in HIV transmission.

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Viral & Immune Dynamics in an NHP Model of Pediatric HIV Infection: Studies to Inform Cure Strategies

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HIV-1 infection in the setting of breastfeeding transmission commits infants to lifelong ART, as interruption is typically followed by return of replication and repopulation of reservoirs. A better understanding of the anatomic origin and kinetics of viral rebound during analytical treatment interruption (ATI) could inform the development of alternatives to ART-based strategies that achieve long-term viral remission in the pediatric population. In this study, sixteen 4-week-old rhesus macaques were orally administered SHIV.CH505.375H.dCT and placed on daily ART at 8 wpi. Median viral loads at peak and just prior to ART were $5x10^5$ and $1x10^5$ copies/mL, respectively. Cell-associated viral DNA was detectable in blood and lymph node CD4+ T cells pre-ART and declined in both compartments by up to 2 logs after ART initiation. SHIV DNA was also detected in gut CD4+ T cells, where higher levels were associated with an increased frequency of gut CCR5+CD4+ T cells at 16 wpi ($p<0.0001$). Consistent with observations from HIV-infected children, SHIV-infected infant macaques showed little to no decline in peripheral CD4+ T cell frequencies during acute infection. Env-specific gp120 and gp41 binding antibodies were detected at 3-4 wpi and displayed a gradual significant decline after ART initiation but remained detectable after several weeks on ART. Recently, we interrupted ART in five animals after 1 yr of treatment to assess viral rebound kinetics. To date, four animals have rebounded within 10-17 d post-interruption with initial viral loads of 10^2-10^5 copies/mL. Continued monitoring of viral and immune parameters during ATI is underway. In summary, we have begun to characterize viral and immune dynamics in a pre-clinical NHP model that displays key features of pediatric HIV infection and uses a chimeric SHIV expressing clade C HIV Env, which allows investigation of HIV envelope-targeting strategies against a subtype highly relevant to the current epidemic.

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HIV-Specific Chimeric Antigen Receptor T-Cells Expand and Persist Following Antigen Boost in Infected, Suppressed Rhesus Macaques

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Background: An effective strategy to achieve antiretroviral therapy (ART)-free remission in HIV⁺ individuals will likely require active approaches such as chimeric antigen receptor (CAR) T-cells, and passive approaches such as CCR5 gene editing. We have modeled an upcoming clinical study in nonhuman primates (NHPs) infected with simian/human immunodeficiency virus (SHIV) and suppressed by ART. We evaluated this approach in stably suppressed animals before and after analytical treatment interruption (ATI). **Methods**: SHIV-infected rhesus macaques (n = 4) were suppressed on ART for at least 1 year prior to intervention. Autologous T-cells were gene-edited with NHP CCR5 CRISPR ribonucleoprotein complexes, then transduced with lentiviral vectors expressing a CD4-based CAR molecule (CD4CAR). Modified cell products were infused without a conditioning regimen. To boost the persistence of these cells *in vivo*, transplanted animals received a single dose of cell-associated viral envelope antigen. Functional assays and flow- and PCR-based measurements were used to quantify the persistence of the T-cell infusion product in blood and tissues. **Results**: Augmented CD4:CD8 ratios increased the persistence of CAR⁺ CD4 lineages *in vivo*. Pre-infection cell sources were more efficiently modified relative to cells from post-infection, ART-suppressed sources. Infusion of cell-based antigen was well-tolerated and led to significant increases in the percentage of CAR⁺ T-cells in peripheral blood. Animals are currently in ATI phase, which initiated 1 month after T-cell infusion. **Conclusions**: Previous studies have demonstrated low-level persistence of virus-specific CAR T-cells *in vivo*. To our knowledge, ours is the first study to successfully boost virus-specific CAR T-cells in infected, suppressed hosts. Animals are currently under close ATI monitoring for multiple parameters including viral rebound kinetics and concordant expansion of CAR⁺ cells. Our approach is highly translatable due to the lack of a cytotoxic conditioning regimen, underscoring the promise of virus-specific CAR-based therapies for viral reservoir reduction in HIV⁺ individuals.

Session IV: Therapeutics/Cure in the NHP Model

Co-Chair: Dr. Luis Giavedoni

Inactivation of proviral SIV DNA with different forms of Cas9

SIV infection in Rhesus macaques results in lifelong infection due to the establishment of reservoir cells that harbor the viral genome integrated in the host genome as proviral DNA. CRISPR/Cas9 reagents can manipulate cellular genomes, including the possibility of inactivating integrated proviral DNA. We designed RNA-guided Cas9 nucleases (RGNu), nickases (RGNi), and inactive Cas 9 (RGNd) targeting conserved regions of the SIVmac239 genome, with the goal of disrupting infectious virus production via insertions and deletions (indels), or RNA interference. We tested these constructs in 2C11 cells, a clonal T-lymphoblast cell line chronically infected with SIVmac. 2C11 cells were nucleofected with plasmids, cells were sorted based on GFP expression, cultured for 15 days, and their DNA was sequenced. Inhibition of SIV by RGNu and RGNi was high and effective even when Cas9 variants were no longer detectable. On the other hand, SIV inhibition by RNAi required constant expression of the gRNA and dCas9; co-expression of the KRAB motif improved SIV RNA silencing. In summary, our multiplex RGNu and RGNi plasmids can inhibit viral replication with very few off-target effects, and are more effective that inhibition mediated by RNA interference.

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Single dose post-exposure treatment with bNAb cocktail achieves clearance of SHIV in infant macaques

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Mother-to-child transmission is the major route of human immunodeficiency virus (HIV) infection for children, and treatments for newborns are needed to abrogate infection or limit disease progression. Previously we showed that short-term broadly neutralizing antibody (bNAb) treatment given within 24 hours of oral exposure cleared highly pathogenic simian-human immunodeficiency virus (SHIV-SF162P3) in a macaque model of perinatal HIV infection. In the current study, all infants treated with a single dose cocktail of bNAbs at 30 hours or a 21 day abbreviated course of ART at 48 hours were aviremic after treatment cessation, with no rebound and only rare occurrences of viral DNA in tissues at necropsy. In contrast, treatment with bNAbs beginning at 48 hours resulted in half of the animals exhibiting tight control, and treatment with ART beginning at 72 resulted in rapid rebound in 5/6 infants. Regardless of treatment regimen, tight controllers did not mount long-lived adaptive immune responses and did not become viremic after $CD8^+$ T cell depletion, suggesting viral control by other mechanisms. We conclude that post-exposure prophylaxis for infant macaques within 30-48 hours of oral SHIV exposure is highly effective using either bNAbs or ART, but that both therapeutic regimen and timing of treatment initiation influence virological and immunological outcomes. Our findings suggest that optimizing the regimen used in clinical intervention may extend the narrow window of opportunity for preventing perinatal HIV infection in the setting of delayed treatment and show that viral clearance with a single dose of bNAbs or a short course ART is an achievable outcome.

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Directed homing of SIV-specific TCR-engineered T cells to B cell follicles through ectopic expression of CXCR5

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The persistence of virus reservoirs in chronically infected HIV+ individuals has long been a barrier to effective cure strategies. B cell follicles in secondary lymphoid tissues are a source of persistent virus during suppressive antiretroviral therapy, in part due to the inability of antiviral CD8 T cells to effectively traffic to this site. We previously demonstrated directed homing of CD8 T cells into B cell follicles through engineered expression of the homing receptor CXCR5 (Ayala et al 2016). To extend this work and direct antiviral T cells into these AIDS-virus sanctuaries, we developed a retroviral vector which expresses both a highly effective TCR specific for the Mamu A*01 SIV Gag CM9 epitope to provide antiviral function, and CXCR5 to direct this response to B cell follicles. Following adoptive transfer, infused T cells were detected in various tissue compartments by flow cytometry and histology. Histological analysis showed specific B cell follicle localization of infused T cells. Recovery of functional engineered T cells from tissues after infusion was demonstrated by *ex vivo* detection of IFNg, MIP1b and CD107a expression following antigen stimulation. Current work is evaluating whether specific cytolytic activity was preserved in infused cells that were re-isolated and expanded *ex vivo*. Taken altogether, our results provide a proof of principle for the engineering of therapeutic virus-specific CD8 T cells and their directed trafficking to sites of viral persistence.

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CD8+ T cells provide limited control of SIV reactivation after combination antiretroviral therapy (cART) cessation

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BACKGROUND: Here we sought to determine the contribution of CD8+ T cell responses in controlling SIV reactivation after cART cessation by performing CD8β depletion to specifically deplete classical MHC 1a-restricted CD8αβ T cells in cART suppressed-SIV-infected rhesus macaques (RM), while leaving non-classical CD8α populations (e.g., NK cells, γδ T cells) unchanged. We also used the barcoded SIVmac239M, which contains at least 10^4 genetically distinct but phenotypically identical viruses, to determine the impact of CD8β depletion on the clonal composition of reactivating infections. **METHODS**: 20 RM were intravenously (IV) inoculated with SIVmac239M prior to cART (tenofovir/emtricitabine/dolutegravir) initiation 12 days post-infection. After 205 days of cART, RM were randomized into two groups (n=10 each) and administered with 6 biweekly doses of an anti-CD8β monoclonal antibody or isotype matched control, IV at 50mg/kg prior to cART release, and a further 3 doses following cART cessation. Plasma viral loads were quantified by qRT-PCR and distinct barcoded viruses quantified by next generation deep sequencing. Lymphocyte population dynamics were evaluated by flow cytometry. **RESULTS**: Anti-CD8β induced a profound depletion of CD8+ T cells in blood, lymph node and bone marrow in all but 3 RM. Upon cART release, all RM rebounded in <12 days and no effect of CD8β depletion on the time to viral rebound, as measured by viremia onset, was observed. There was also no effect of CD8β depletion on the rate of rebounding variants detectable in plasma. However, RM in which CD8+ T cells were effectively depleted showed a 2-log increase in peak and set point viremia. **CONCLUSIONS**: Collectively, these results indicate that although CD8+ T cell responses have a definitive effect on viral load set points after cART release, these responses are too late or not of sufficient magnitude and/or potency to prevent or delay rebound to mediate stringent post-cART control.

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Virologic response to concomitant administration of SIV-specific engineered T cells and heterodimeric IL-15 in a SIV-infected Rhesus macaque

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Adoptive T cell therapy (ACT) holds promise to enhance immune control of tumors and infectious diseases, but requires timely delivery and persistence of sufficient numbers of functional engineered cells in the appropriate tissues to improve disease outcomes. We developed retroviral vectors for transduction of primary rhesus macaque T cells to enable expression of the chemokine receptor CXCR5, demonstrating effective localization in B cell follicles of secondary lymphoid tissues following infusion (Ayala, et al, J Virol, 2017), and vectors for co-expression of CXCR5 with SIV-specific T cell receptors. Heterodimeric IL-15 enhanced persistence and effector function of adoptively transferred T cells in a murine cancer model (Ng et al, Clin Cancer Res, 2017), and showed antiviral activity in SHIV-infected rhesus macaques (Watson et al, PLoS Pathog, 2018). We combined treatment with autologous T cells engineered for co-expression of MamuB*08-restricted SIV specific TCRs and CXCR5 with administration of heterodimeric recombinant rhesus IL-15 (RhhetIL-15) in a Mamu B*08+ rhesus macaque partially controlling SIVmac239-infection. Infused cells showed robust in vivo proliferation in peripheral blood based on fluorescent dye dilution and post-infusion lymph node (LN) biopsies showed proliferating infused cells localized to LN, including in B cell follicles. The combined treatment was associated with a dramatically decreased frequency of SIV RNA+ cells in LN, and decreased plasma viremia, which reached a nadir of 2 logs lower than pre-treatment levels by 17 days post-infusion. As persistence of the infused cells and effects of the administered RhHet-IL-15 waned over the weeks following infusion, plasma viremia gradually returned to pre-treatment levels. These encouraging results provide impetus for detailed expanded studies to clarify the independent and combined activities of the RhHet-IL-15 and the infused engineered cells, and to optimize treatment schedules to achieve durable virologic impacts.

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Control of virus replication in SIV-infected rhesus macaques after therapeutic vaccination is associated with polyfunctional SIV specific T-cells localized in the mesenteric lymph nodes.

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Background: We previously showed that a therapeutic SIV DNA vaccine, adjuvanted with *E. coli* heat labile enterotoxin (LT), achieved durable viral control in 50% of infected macaques. Here, we evaluated the potential for a novel combination of potent adjuvants, including LT, to enhance efficacy and characterized immune responses associated with viral control post-cART. **Methods:** Rhesus macaques were infected with SIVΔB670, started cART 6 weeks post-infection (wpi), and received 5 therapeutic immunizations spaced 4 weeks apart beginning at 32wpi. Subsequently, cART was discontinued at 55wpi to assess efficacy. One group received a multi-antigen DNA vaccine (MAG) expressing SIV Gag, Pol, Env and Nef, adjuvanted with LT (MAG+LT; N=5). Another group received MAG formulated with a genetic adjuvant cocktail expressing sCD80, sPD-1, IL-12, IL-33, RALDH2, and LTA1 (MAG+AC; N=5). Controls received empty plasmid DNA (Controls; N=4). T-cell responses in PBMC and mesenteric lymph node (MLN) were measured by flow cytometry and viremia was measured by RT-PCR. **Results:** All animals exhibited robust viremia during acute infection (median 10^7 RNA copies/mL plasma), but viral replication was not fully suppressed by cART. Post-cART, 3/5 MAG+AC animals sustained viremia below 10^3 RNA copies/mL plasma, compared to 1/5 MAG+LT and 1/4 control animals. Although there were no statistical differences in efficacy between groups, lower viral burden post-cART among controllers significantly correlated with triple functional $CD8^+$ T-cells (P=0.006), in particular $CD8^+IL\text{-}2^+IFN\gamma^+TNF\alpha^+$ T-cells (P=0.04), in MLN but not PBMC. **Conclusions:** Despite suboptimal viral suppression on cART, a novel adjuvant combination showed promise in improving therapeutic vaccine efficacy. Protection from viral rebound was associated with quality of SIV-specific immune responses localized in MLN but not blood. These results indicate that gut mucosal immune responses play a key role in containing residual virus post-ART and highlight the need for therapeutic vaccines/adjuvants that can restore functional quality of mucosal T cell responses.

Session V: Vaccine Development

Co-Chair: Dr. Margareth Ackerman

Learning important immune connections: correlates of protection across studies and species

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Antibodies act as a nexus between innate and adaptive immunity: they provide a means to target the potent toxic activities of a spectrum of innate immune effector cells in order to clear viral particles and infected cells. This functional landscape is remarkably complex, with multiple antibody variants directed to multiple epitopes on multiple viral antigens. This diversity of viral recognition characteristics is further complemented by diversity in each antibody's ability to recruit the potent anti-viral effector functions of a suite of innate immune effector cells such as Natural Killer cells and phagocytes. Results from both human and animal model studies have implicated these effector functions in vaccine-mediated protection, but the complexity of these activities has challenged traditional methods of evaluating antibody responses. We demonstrate how high-throughput, high-content platforms for the biophysical and functional interrogation of the innate immune recruiting capacity of diverse virus-specific antibodies are capable of parsing this complex molecular landscape into components that can be used to develop computational models of antibody activity and provide insights into mechanisms of vaccination and new prospects for engineering innovative antibody therapies. This talk will focus on insights resulting from applying artificial intelligence to learn from humoral immune profiles to better understand mechanisms of antibody-mediated protection from HIV infection in humans and animal models.

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Single-cell cloning of natural killer cells for in depth analysis of adaptive functions against HIV/SIV

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Beyond their ability to eliminate infected cells without the need for prior sensitization, natural killer (NK) cells were shown to exhibit some adaptive immune functions. In particular, our lab demonstrated that antigen-specific memory NK cells responses against SIV and rhCMV can be induced by infection and vaccination in non-human primates (NHP). These suggested that a subset of NK cells in humans may also mediate immunological memory responses to HIV. To study HIV-antigen-specific NK cells in more detail, we performed limiting dilution assays or single-cell sorting to clonally expand single NK cells (NKCL) from HIV-infected individuals in the presence of irradiated feeders and high-dose IL-2. Both techniques generated at least 10-20 NKCL per individual, each NKCL growing to reach over five million cells by 4-5 weeks of culture. Sorting has the advantage of isolating NK cells expressing specific receptors to be tested for their involvement in antigen-specific responses. Matched lymphocytes were used to generate B-LCL using EBV transformation. Using a calcein acetoxymethyl ester (CAM) cytotoxicity assay, the ability of each NKCL to lyse K562-cell lines or autologous B-LCLs stained with CAM and pulsed with different HIV-derived and control peptide pools was assessed. Our procedure showed for the first-time antigen-specificity for HIV among human NK cells. All tested NKCL could potently lyse K562 cells (61%) and killing of control peptide-pulsed B-LCL was minimal (<8%). Strikingly, 27% of NKCL displayed anti-Gag and/or anti-Env cytotoxic activity up to 87% killing, and was dependent on NKG2 expression as determined by antibody blocking. We are working to modify cloning procedures to isolate analogous SIV-specific NK cells from NHP using different sources of feeders and cytokines. So far, we were able to expand single NHP cells with innate characteristics. Collectively, single-cell cloning could elucidate key information on antigen-specific NK cells features to be harnessed in future therapies.

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Protective gene expression signature of the RhCMV/SIV vaccine

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The simian immunodeficiency virus (SIV)-targeted vaccine vectors based on rhesus cytomegalovirus (RhCMV) strain 68-1 elicit potent cellular immune responses that can fully protect rhesus macaques (RM) against SIV infection. Thirty RM were vaccinated with the 68-1 vaccine via subcutaneous or oral delivery. Following vaccination, animals were subjected to repeated limiting dose intrarectal SIVmac239 challenge until infected by either detection of plasma virus or de novo development of T cell responses to SIVvif. Regardless of delivery mode, over half of the vaccinated animals manifested stringent aviremic control of the virus. To define the molecular features of the protective 68-1 vaccine response, we performed mRNA-seq assays on whole blood samples collected during the vaccination phase. Bioinformatics analyses compared the transcriptional profiles between the protected and non-protected animal groups. Differences in these RNA profiles included magnitude and directionality of differentially expressed genes involved in several innate immune networks and specific cytokine response networks, featuring a novel IL-15 signature, impacting innate immune activation, inflammation, and immune programming. Examination of the gene expression from blood of IL-15-treated animals reveals genes correlated to vaccine protection within the IL-15 response program. Statistical-driven computational analyses, rule-based modeling, and multivariate statistical analyses of each signature has defined the significant gene and gene network correlates of protection, and identified a subset set of genes that serve to predict vaccine efficacy outcome. The defined gene signatures for both protected and non-protected animals are being applied toward efforts to understand the mechanisms responsible for the unique “control and clear” efficacy manifested by the 68-1 RhCMV vectors, and to improve vaccine efficacy for complete protection across vaccinated individuals. Lessons from these studies will be applied to the development of human CMV vaccine vectors designed to translate the CMV vector approach for vaccination of humans for protection against HIV infection.

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Rhesus macaques co-immunized with influenza and SIV DNA vaccines develop similar responsiveness to both vaccines

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Responses to SIV and HIV vaccines are highly variable. Multiple factors such as age, genetics, and sex can influence vaccine responses, but it is unclear if this variability is driven by host factors or the immunogen used. Here, we investigated immunogenicity and protective efficacy of SIV vaccination and concurrently, immunogenicity of influenza vaccination in the same animals. Twenty-two rhesus macaques were stratified to receive both influenza and SIV vaccination (n=14) or influenza vaccination alone (n=8). Animals were vaccinated by intra-dermal electroporation at weeks 0, 4 and 17 with universal influenza A +/- SIV rhFLSC$_{smE660}$ DNA vaccines. To avoid antigen competition, influenza and SIV vaccines were administered to the skin at different anatomical sites. Vaccine-specific T-cell responses were measured in mucosal tissues and blood 2 weeks after final vaccination by IFNγ-ELISpot and intracellular staining. Macaques were challenged with up to 10 weekly intrarectal low-doses of heterologous SIVmac251 starting 8 weeks after final vaccination until confirmed SIV infected. As expected, we observed variability in responsiveness to both vaccines. Interestingly, in influenza/SIV co-immunized animals the SIV and influenza CD8+ T-cell responses correlated significantly (Spearman r=0.61, p=0.02), indicating host-intrinsic factors influenced vaccine responses. Immunized animals developed mucosal responses including influenza-specific lung and SIV-specific jejununal T-cell responses. Following SIV challenge, viral loads of vaccinated animals were only ½ log lower than controls. However, viral set-point was variable (3-log spread), and jejunum T-cell polyfunctionality significantly correlated with lower SIV viral burden, peak and set-point (Spearman r=-0.55,-0.49,-0.45, p=0.01,0.02,0.03 respectively), suggesting gut mucosal T-cell responses enhanced viral control. Collectively, these results indicate a role for vaccine-induced mucosal immune responses in protection and induction of these responses by skin-delivered DNA vaccines. These results also reveal host-intrinsic factors may hamper consistent vaccine immunogenicity. Additional studies are needed to define these host factors and develop vaccine strategies to overcome these barriers.

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Adjuvant-dependent Modulation of CD4 T Follicular Helper Cells Impacts Longevity and Functional Quality of Antibody Responses to a subtype C HIV-1 Envelope Vaccine in Rhesus Macaques

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Generating durable humoral immunity through vaccination depends upon effective interaction of follicular helper T cells (T_{fh}) with germinal center (GC) B cells. T_h1 polarization of T_{fh}cells is an important process shaping the success of T_{fh}-GC B cell interactions by influencing co-stimulatory and cytokine-dependent T_{fh}help to B cells. However, the question remains whether adjuvant-dependent modulation of T_{fh}cells enhances HIV-1 vaccine-induced anti-Envelope (Env) antibody responses. We investigated whether an HIV-1 vaccine platform designed to increase the number of T_h1-polarized T_{fh}cells enhances the quality and longevity of anti-Env antibodies. Utilizing a novel interferon-induced protein (IP)-10-adjuvanted HIV-1 DNA prime, followed by an MPLA+QS-21-adjuvanted Env protein boost in macaques (T_h1 group), we observed higher anti-Env serum IgG titers with greater cross-clade reactivity, specificity to V1V2, and effector functions when compared to macaques primed with DNA lacking IP-10 and boosted with MPLA+alum-adjuvanted Env protein (T_h1+2 group). The T_h1 vaccine regimen elicited higher anti-Env IgG1 and lower IgG4 antibodies in serum, showing for the first time that adjuvants can dramatically impact the IgG subclass profile in macaques. The T_h1 regimen also increased vaginal and rectal IgA antibodies to a greater extent. Within lymph nodes, we observed augmented GC B cell responses and promotion of T_h1 gene expression profiles in GC T_{fh} cells. The frequency of GC T_{fh} cells predicted both the magnitude and avidity of anti-Env serum IgG. Together, these data suggest that adjuvant-induced stimulation of T_h1-T_{fh} cells is an effective strategy for enhancing the longevity and quality of anti-Env antibody response.

Session V: Vaccine Development

Co-Chair: Dr. Marie-Claire Gauduin

Epithelial Stem Cell-based AIDS Vaccine to Induce Mucosal Immune Responses and Protection against SIV Challenge in Macaques

A vaccine that restricts viral replication at mucosal entry may control HIV transmission. We used the epithelial stem cells as permanent source of viral antigens and their differentiated offspring as antigen-presenting cells. We developed a single-cycle SIV vaccine under the control of the involucrin promoter, which was tested for its ability to drive SIV expression in terminally differentiated epithelial cells, induce mucosal responses and protect against challenge. Sixteen macaques (8 females, 8 males) were immunized (1 dose, atraumatic) at week 0 and monitored for specific immune responses in blood, mucosal secretions, various lymphoid/non-lymphoid tissues. Within 2-weeks post-vaccine strong mucosal antibody responses (IgG, IgA) and specific CD8+ cells expressing a4ß7 were detected. Animals were challenged at weeks 12 or 24 using repeated low-doses SIVmac239. Eight additional macaques served as unvaccinated SIV-infected Controls. Repeated low-dose challenges revealed significant delay or lower viremia with 2-3 logs reduction at peak, 4-5 logs-reduction at set-point, to undetectable viremia by week 20 post-SIV in animals vaccinated animals. Interestingly, two males were protected as early as 12-weeks post-vaccine with no detectable virus in blood or tissues/organs. Controls had high viremia (log10: 7.2-8.7 viral RNA copies/ml, peak) and significant gut CD4+ T cells depletion. We demonstrated a positive correlation between mucosal and systemic T cell responses and control of viremia; and, an inverse association between viremia and post-challenge vaginal antibody responses. Vaccinated animals manifested durable control of infection for 2 years when CD8-depletion was performed. The dramatic fall in viremia coincided with the recovery of T cells in blood and the significant increase of systemic IgG and SIV-specific CD8+ cells. We demonstrated the efficacy of an epithelial stem cell-based vaccine to serve as antigen delivery system to generate specific immune responses leading to better protection or significant delay in infection, and rapid control of viremia to undetectable.

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A vaccination regimen to enhance immune responses associated with reduced risk of mother-to-child transmission of HIV

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Maternal anti-retroviral therapy (ART) can reduce the risk of mother-to-child transmission (MTCT) to as low as 2%. However, failures in adherence and implementation of ART hinder progress towards the elimination of pediatric HIV infection, suggesting a need for maternal vaccination strategies that synergize with current ART regimens. We previously reported that maternal envelope (Env) variable loop 3 (V3) - specific IgG, CD4-blocking, and tier 1 neutralizing antibodies were associated with a reduced risk of MTCT of HIV in a cohort of US Mother-Infant pairs. Additionally, V3-specific antibodies isolated from a non-transmitting, HIV-infected mother neutralized her own circulating virus, suggesting that antibodies with autologous neutralization capacity are protective in the setting of MTCT. Our goal was to evaluate the immunogenicity of a vaccine regimen to boost potentially protective HIV Env-specific antibody responses in SHIV-infected, ART-suppressed, non-pregnant female rhesus macaques (RMs). Twelve female RMs were infected intravenously with SHIV.C.CH505. Twelve weeks post-infection (wpi), RMs began a daily ART regimen. At 2, 6, and 10 weeks of ART, RMs received either a HIV clade B/C gp120 (n=6; vaccine group) or RSV (n=6; control group) vaccine intramuscularly. ART was discontinued after 12 weeks and RMs were monitored for viral rebound and antibody responses. While autologous virus neutralization responses were similar between both groups, HIV gp120 vaccination was able to enhance Env-specific IgG binding and ADCC responses specific for the Env vaccine immunogens and challenge virus. Additionally, the vaccinated cohort exhibited enhanced V3-specific and soluble CD4-blocking IgG responses, and a rapid increase in tier 1 virus neutralization responses, all previously identified predictors of MTCT risk. Thus, vaccination of SHIV-infected macaques in the setting of ART not only induces robust vaccine-specific antibody responses, but may stimulate antigenic imprinting to boost Env-specific responses to the challenge antigen, that could be harnessed for further reducing MTCT risk.

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CMV vaccine vector-induced protection against SIV in Mauritian cynomolgus macaques

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Strain 68-1 rhesus cytomegalovirus (CMV) vaccine vectors expressing simian immunodeficiency virus (SIV) antigens (RhCMV/SIV) prime broadly-targeted, unconventionally MHC-II- and MHC-E-restricted CD8+ T cell responses that stringently control SIV replication in vaccinated rhesus macaques (RM). However, RM express many more MHC-II and MHC-E alleles than humans, and it remains unclear if the unprecedented cellular immunity and control of SIV observed in RhCMV/SIV-vaccinated RM is due to the unique immunogenetics of RM or species-specific functions of RhCMV itself. In contrast to RMs, Mauritian cynomolgus macaques (MCM) exhibit reduced genetic diversity with immunogenetics that more closely resemble those of humans. However, 68-1 RhCMV was unable to elicit unconventionally restricted CD8+ T cells in MCM suggesting a species barrier for viral vector function. To determine if non-classical T cell priming and protection against mucosal SIV challenge is restricted to RhCMV-vaccination of RM or a universal phenomenon, we vaccinated eight MCM with a '68-1 like' cynomolgus CMV expressing SIV antigens (CyCMV/SIV). CyCMV/SIV vaccinated MCM generated unconventionally, MHC-II- and MHC-E-restricted T cell responses comparable to RhCMV/SIV vaccinated rhesus macaques. Upon repeated, limiting-dose, intrarectal challenge with SIVmac239, 50% of CyCMV/SIV vaccinated MCM stringently controlled SIVmac239 replication, defined as no plasma viremia and the development of T cell responses against SIV proteins absent from the vaccine. Acquisition and subsequent control of SIV was confirmed by cell-associated viral loads and adoptive transfer to naïve MCM of tissue biopsies from CyCMV/SIV-protected animals. Thus, we have confirmed the distinct immunologic and protective phenotype induced by CMV vaccines in a second nonhuman primate species with immunogenetics reflective of humans, indicating that these results are not unusual species-specific traits of RM or RhCMV and that 68-1 like HCMV/HIV vaccines might similarly recapitulate unconventional T cell restriction and protect against HIV.

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Contribution of vaccine-induced mutant epitope-crossreactive CD8+ T cells to stable control of SIVmac239 replication

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We have been working on $CD8^+$ T cells targeting Mamu-A1*065:01- restricted SIV $Gag_{241-249}$ epitope, which is located in a region corresponding to the protective HLA-B*58:01-restricted HIV $Gag_{240-249}$ TW10 epitope. Rhesus macaques inducing this single $Gag_{241-249}$ epitope-specific $CD8^+$ T cells by vaccination controlled setpoint viremia after an SIVmac239 challenge (J Virol 83:9339, 2009). Some of these SIV controllers accumulated proviral $CD8^+$ T-cell escape mutations during viral control and showed reappearance of plasma viremia more than 1 year post-infection, whereas others maintained stable SIV control without detectable $CD8^+$ T-cell escape mutation (PLoS Pathog 11:e1005247, 2015). Here, we compared vaccine-induced $Gag_{241-249}$-specific $CD8^+$ T cells in the former transient SIV controllers and the latter stable controllers. Based on our previous observation that $Gag_{241-249}$-specific $CD8^+$ T-cell responses select for a viral escape mutation resulting in D to E change at Gag residue 244 (J Virol 86:6481, 2012), we used the wild-type $Gag_{241-249}$-Mamu-A1*065:01 tetramer and the mutant $Gag_{241-249}$.244E-Mamu-A1*065:01 tetramer in this study. Analysis of rhesus macaques vaccinated with a Sendai virus vector expressing EGFP-$Gag_{236-250}$ fusion protein revealed that some vaccinated animals induced $Gag_{241-249}$-specific $CD8^+$ T cells detected only by wild-type tetramer but not by mutant (referred to as non-crossreactive), whereas others induced $Gag_{241-249}$-specific $CD8^+$ T cells detected by mutant tetramer as well as wild-type (referred to as crossreactive). Interestingly, after SIVmac239 challenge, the former vaccinees inducing non-crossreactive $Gag_{241-249}$-specific $CD8^+$ T cells exhibited the pattern of transient SIV control with accumulation of proviral $CD8^+$ T-cell escape mutations, but the latter inducing crossreactive $Gag_{241-249}$-specific $CD8^+$ T cells showed the pattern of stable SIV control without detectable $CD8^+$ T-cell escape mutation. These results indicate the contribution of vaccine-induced crossreactive $Gag_{241-249}$-specific $CD8^+$ T cells to stable SIV control, implying the rationale for induction of mutant epitope-crossreactive $CD8^+$ T cells by vaccination toward stable HIV control.

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Efficacy of Novel Recombinant Simian Varicella Virus-Simian Immunodeficiency Vaccines, rSVV-SIV, Following Mucosal SIV Challenge in the Rhesus Macaque

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Developing an effective AIDS vaccine remains one of the highest priorities in HIV research. The live attenuated varicella-zoster virus vaccine, a known safe and effective chickenpox/zoster vaccine, is a potential recombinant vaccine against other pathogens, including HIV. Using the simian varicella virus (SVV) counterpart virus, novel recombinant SVV-SIV vaccines expressing SIV gag and env genes were tested in rhesus macaques for induction of SIV-specific immune responses and protection against mucosal SIV infection. Fifteen female rhesus macaques were divided into Vaccine n=8 and Control n=7. The Vaccine macaques received subcutaneous and intranasal immunizations with rSVV-SIV*gag* and rSVV-SIV*env* at d0, 8, 24, 43 and 55 weeks and were boosted with 100ug SIVenv and SIVgag proteins in Adjuplex® at 24, 43 and 55 weeks. Controls were similarly immunized with rSVV-RSVg and boosted with PBS in Adjuplex®. Immunizations were well tolerated and produced strong SIV-specific humoral and cellular immune responses. SIV neutralizing antibodies against the Tier 1 SIV challenge virus were also produced. Following immunizations, all animals were intravaginally challenged weekly with pathogenic SIVmac251. Plasma viral loads (VL) were monitored with RT qPCR. Thirteen weekly intravaginal SIVmac251 CX-1 challenges (316 - 1,264 $TCID_{50}$) resulted in infection of all 7 (100%) Controls, while only 5 of 8 (62.5%) Vaccine animals were infected. The 5 infected Vaccine animals showed significantly lower VLs with the peak mean VL reduced two logs from Controls (p=0.04). Three (37.5%) Vaccine animals remained uninfected and protected. Our results demonstrate recombinant varicella-SIV vaccines with protein boosts strongly stimulated humoral, cellular and neutralizing immune responses against SIVmac251. Following SIV mucosal challenge the rSVV-SIV vaccines provided protection of 3 of 8 animals, and of the 5 of 8 Vaccine animals infected, resulted in a significantly reduced peak VL. These results suggest strong potential for these novel rSVV-SIV vaccines to protect against mucosally transmitted SIV.

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Sequence and structure guided HIV-1 Clade C trimeric immunogen design to induce neutralizing and V1V2 directed antibody responses

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About 50% of global HIV-1 infections are due to clade C viruses and there is a great need for the development of stabilized native-like trimeric clade C gp140 protein immunogen for inducing neutralizing antibodies by vaccination. The C.1086 based gp140 trimer would be of interest as the monomeric gp120 version of this protein is currently being used in a Phase 2a/b clinical study (HVTN702). The unstabilized C.1086 (K160N included in all constructs to improve binding to bnAb PG9) gp140 protein does not induce autologous neutralizing antibodies. To develop a stable trimer, we adopted recent structure guided strategies to design SOSIP, NFL (Native Flexible Linker) and UFO (Uncleaved Full-Length Optimized) forms of the protein. The NFL and UFO versions yielded higher trimeric fractions than the SOSIP counterpart which predominantly formed aggregates. UFO design was further selected based on improved binding to V1V2 specific bnAb PG16 than C.1086 K160N NFL. Sequence guided mutational analysis of the V2 hotspot region (V2HS,165-181) highlighted K166R to markedly improve binding to the V1V2 trimer-specific bnAb PGT145. Additional structure guided modifications were adopted to improve the stability of the envelope. A variant at V2HS; UFO-v2-HS showed significant enhancement in binding to multiple V1V2 directed bnAbs and increased the trimeric fraction by 20%. Following immunization of rabbits, UFO-v2-HS induced higher gp70-V1V2 specific and membrane anchored trimeric envelope specific binding antibodies compared to wild type gp140 immunized animals. Some of the immunized rabbits also induced autologous tier-2 neutralizing antibodies up to a titer of 535. We are currently analyzing the neutralization specificity of the serum. Encouragingly, UFO-v2-HS immunized rhesus macaques also elicited strong autologous neutralization titer (100-800). These results demonstrate that the stabilized C.1086 K160N UFO trimer protein can induce autologous tier-2 neutralizing antibodies and enhance binding antibodies specific to gp70-V1V2 and membrane anchored trimeric Env.

Poster Abstracts

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Heterogeneity of the active reservoir size in the lymph node of cynomolgus macaques latently infected with a macaque-tropic HIV-1 (HIV-1mt)

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It has been shown that approx. 0.3% of the HIV-1-infected individuals lead to elite controllers (EC); EC is mostly durable but is not necessarily long-lasting. Accumulating evidence indicates that 30% of EC lose the control and develop the viral persistency; however, it remains unclear how the durability of EC is determined. We previously reported in this symposium that a cynomolgus macaque infection of macaque-tropic HIV-1 (HIV-1mt) with CCR5-Env. This macaque model has distinctive characteristics; (i) the HIV-1mt-challenged macaques mostly become durable latency without detectable PVLs, while some cases of persistent viremia are also observed, (ii) the latency is immunologically controlled, and (iii) follicular helper T (Tfh) cells harbor replication-competent HIV-1 and the small fraction of cells produce unspliced viral RNA as active reservoirs. We further examined the underlying mechanism by which the loss of control was established and found that four of nine latently infected macaques exhibited higher CA-RNA levels in the lymph node (LN) lymphocytes (median; $>10^3$ copies per 10^6 cells), while other five individuals had lower CA-RNA levels (median; <20 copies per 10^6 cells). The difference in the proviral DNA levels between the two groups was only less than 10-fold, indicating efficient viral replication in the former group. Importantly, the former group included the two latency monkeys, who exhibited spontaneous loss of control and viral persistency in parallel with extensive viral escape/adaptive mutations. Collectively, our results in this study suggest that greater active reservoir size in LN may be associated with the loss of control in our latency macaque model. Evaluating relative value of CA-RNA per proviral DNA levels in LN may be valuable to see if it might be a good marker for predicting loss of control in natural controller including EC.

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Detection of Cytomegalovirus in saliva from rhesus macaques in an expanded SPF derivation colony at Tulane National Primate Research Center.

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Worldwide, cytomegalovirus (CMV) is the most common pathogen transmitted congenitally in humans. Congenital CMV infection in infants can produce lifelong neurological sequelae, as well as severe disease from primary infection or viral reactivation from latency in immunocompromised hosts. Understanding the virus life cycle and determinants of infection would benefit vaccine development. Rhesus macaques represent an optimal animal model for CMV studies due to the high similarity between rhesus CMV and human CMV as well as pathogenesis, prevalence, and transmission. In this report, we present results of CMV longitudinal testing of rhesus macaques from the expanded specific pathogen free (eSPF) derivation colony in comparison to the SPF-4 colony at TNPRC. To evaluate the CMV infection status, saliva samples were collected from 84 animals from the eSPF derivation colony between the ages of 12 days and 3 years, and from 48 animals from the SPF-4 colony between the ages of 6 months and 1.5 years. Samples were analyzed by quantitative real time PCR as previously described (Kaur et al., 2002). Results were interpreted as negative (no amplification), indeterminate (< 10 copies) or positive (≥ 10 copies). No positive samples were identified from animals examined from the eSPF derivation colony. A fraction of the samples was identified as indeterminate due to low level of amplification (< 10 copies). However, in all animals with indeterminate results, status of infection was confirmed as negative in at least 2 later consecutive samples, as well as negative serology results by Luminex-based assay and/or indirect immunofluorescence assay. Conversely, 46 of 48 animals from the SPF-4 colony were identified as CMV-infected, with high level of amplification (up to 5 x 10^6 copies). This study confirms that in rhesus macaques early derivation (≤ 3 days post-birth) reduces the exposure to CMV infected animals, thus limiting the establishment of persistent infection.

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Early infancy gut microbiota predicts host anti-drug antibody

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The gut microbiome plays an essential role in the development of the immune system in humans and animals. Microbial colonization at an early age is necessary for the normal development of the immune system and variation in microbial composition can lead to alterations of the immune system, ultimately resulting in an over- or underrepresentation of immunologic cell subunits. Our preliminary results from another study show that peak vaccine-induced immune responses in infant macaques immunized with human immunodeficiency virus envelope vaccines are predicted partially by the early-life intestinal gut communities. In this study, we inoculated neonatal rhesus macaques with recombinant adeno-associated virus vectors for the passive transfer of genes, encoding for HIV entry inhibitor antibody like molecules. The host anti-drug antibody (ADA) levels were evaluated and the early life intestinal microbial communities were profiled using 16S rRNA gene sequencing. Our data suggests that early life gut microbiotas and their predicted metagenomic contents are capable of predicting the host's ADA. Insights on early life microbial composition and its potential effect on the host response to the HIV vaccines or treatments could explain the wide variation in the vaccine/treatment responsiveness among humans and animals. Using the early life gut microbiome as a prediction model for the function of immune responses could possibly lead to new approaches to vaccination protocols and may improve vaccine efficacy and individual vaccine responsiveness in human and veterinary medicine.

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Generation of minimally adapted SHIV.C.CH505 with enhanced viral kinetics

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Our lab developed a novel strategy to generate SHIVs that encode native HIV-1 Envs, all of which successfully infect rhesus macaques (RM). Some SHIVs are controlled to $<10^3$ plasma virus copies/ml in a fraction of RM; SHIV.C.CH505, which encodes an Env of significant interest, was controlled in 3/13 RM by 40 weeks post-infection. Here, we endeavored to generate minimally adapted SHIV.C.CH505 with enhanced replication. Using single genome sequencing of gp160 *env,* we identified a signature of common mutations in plasma virus from 3 SHIV.C.CH505-infected RM with high viral loads (VL). The same signature of mutations was enriched in 5 high VL animals in an independent study of 22 RM. We performed a limited passage experiment in 2 RM to test the hypotheses that (1) the signature mutations would arise early and (2) initiating infection with virus containing these mutations would lead to improved viral kinetics. RM1 was inoculated with a mixture of early plasmas from 3 SHIV.C.CH505-infected RM. RM2 was inoculated with early plasma from RM1. Both RM exhibited high VLs (10^{3-5} copies/ml) for over a year. In both RM, viruses containing the signature mutations arose early and went to fixation. To test the effect of signature mutations in vitro, we infected primary RM CD4 T cells with >15 clones containing combinations of observed mutations. Clones with single and combination mutations demonstrated markedly greater in vitro replication than SHIV.C.CH505. Neutralization assays confirmed minimal changes in the resistance profile of a 5-mutation clone (SHIV.C.CH505.adapted) vs. the parental SHIV. These results suggest that we have generated minimally adapted and antigenically conserved SHIV.C.CH505 with enhanced in vitro viral kinetics. In vivo experiments, including comparing parental vs. adapted SHIV and long-term monitoring of SHIV.C.CH505.adapted-infected RM with and without ART, are ongoing. SHIV.C.CH505.adapted has potential as an NHP reagent with broad applications for HIV research.

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Development of a polyfunctional antiviral broadly neutralizing antibody combination for passive immunization of in Simian Human Immunodeficiency Virus (SHIV)-infected infant Rhesus macaques.

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Despite the success of early combination antiretroviral therapy (ART) in children, the eradication of long-lived latent viral reservoirs remains challenging. Clinical trials and animal studies indicated that broadly neutralizing antibodies (bNAbs) can delay virus rebound. However, these studies did not include children highlighting the need to develop cure strategies adapted to pediatric settings. We hypothesized that passive immunization with a polyfunctional cocktail of bNAbs will delay virus rebound in SHIV-infected infant rhesus macaques. First step to test this hypothesis, we will develop an antibody cocktail capable of mediating robust neutralization and Fc effector functions by combining bNAbs targeting three distinct regions of the HIV Env. We first compared the neutralization sensitivity of a panel of 18 bNAbs against 3 clade C Transmitted/founder SHIVs (SHIV.CH505, CH848, and 1086c) and observed that overall SHIV CH505 was more sensitive to neutralization. Interestingly, there was a strong correlation in the neutralization titers against the SHIVs and their corresponding HIV Env variants ($p<0.0001$ for all), indicating that these SHIVs conserve the neutralization profile of their parental HIV. Based on their ability to neutralize SHIV CH505, we selected seven bnAbs (CD4-binding-site (n=3), V2-glycan (n=3) and interface (n=1) for combination analysis (9 combinations). The top three neutralizing combinations against SHIV.C.CH505 (IC_{80}range 0.02-0.023µg/ml) consisted of PGDM1400 (V2-glycan) combined with PGT151 (interface) and either of the CD4-bs bNAbs (3BNC117, CH235.12 and CH31). The combination of PGDM1400+PGT151+3BNC117 was also among the most potent against other clade C SHIVs (SHIV.CH505, CH848, 1086c and 1157(QNE)-Y173H, IC_{50}range 0.004-0.06µg/ml). In conclusion, we developed a polyfunctional bNAbs cocktail with robust antiviral potency and our data identified PGDM1400+PG151+3BNC117 as a potential candidate for passive immunization studies in infant macaques. In future work, we will expand our virus panel to include cross-clade SHIVs (SHIV.A.BG505 and SHIV.B.SF162P3) and assess the triple combinations ability to mediate ADCC and ADCP.

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Viral gene expression in CSF CD4 T cells during acute SHIV infection of rhesus macaques

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The origin and extent of viral replication within the central nervous system during acute HIV-1 infection remains unclear. We assessed cerebral spinal fluid (CSF) for host cells harboring virus in acutely SHIV-infected macaques and compared infected cell frequency and cellular activation status to that observed in peripheral blood and lymph node mononuclear cells (PBMC, LNMC). Rhesus macaques (n=18) were infected intrarectally with SHIV-1157ipd3N4 and sampled 2-12 weeks post-infection (PI). Cell populations were FACS sorted in replicates. SHIV RNA+ cell frequency was determined by RT-qPCR for unspliced (*gag*) and spliced (*env*) viral RNA and either Poisson distribution statistics (PBMC and LNMC) or assigning one infected cell to positive CSF replicates. Markers of cellular activation were measured by flow cytometry and gene expression. Infected, transcriptionally active (*env+gag+*) CSF CD4 T cells were detected in 25% and 12% of animals at 4 and 12 weeks PI, respectively. In animals with SHIV RNA+ CSF CD4 T cells, infected (*gag+*) cell frequency was similar across CSF (0.05-2%), PBMC (0.02-7%), and LNMC (0.03-2%), indicating comparable T cell infection burden in these compartments. While macrophage infection was infrequently observed in CSF, limited cell numbers constrained sampling depth. Surface expression of CD38 was elevated on CD4 and CD8 T cells in both PBMC and CSF compared to uninfected controls. In contrast, CD169, as well as CD38, was elevated on monocytes/macrophages in PBMC but not CSF, indicating T cell but not macrophage activation in CSF during acute infection. CSF CD4 T cells and macrophages both upregulated *CXCL10* compared to uninfected controls and therefore may contribute to early CSF inflammation. Our data supports a model of active CD4 T cell infection within the CNS during acute HIV/SHIV infection, distinct from the role of macrophages in advanced infection.

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Vaccine-elicited memory B-cells expressing ADCC-mediating antibodies lacking recognition of the V2 region did not correlate with protection of NHPs.

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Background: We have previously reported that a multiclade polyvalent DNA prime/protein boost (UMMS A, B, C, AE gp120 envelopes [Env]) vaccine elicited robust cross-clade ADCC-mediating serum antibody. Serum antibodies against V2 peptide, a specificity that synergized with other ADCC-mediating antibodies in the RV144 trial, were commonly induced. However, no protection from challenge with clade C CH505.TF heterologous virus was observed. Here, we investigated the breadth and specificity of vaccine-induced memory B-cells in three NHPs with strong serum ADCC activity. **Methods:** PBMCs were collected 2 weeks post final boost, before challenge with SHIV CH505.TF. Env immunogen-reactive memory B-cells were sorted and stimulated using our single-cell culture system. On culture supernatants, we measured binding to immunogen Envs, CH505. TF and AE.A244 V2 peptides, CH505.TF-infected cells, and measured ADCC against CH505.TF and the UMMS B Env immunogen. **Results:** From three NHPs, we isolated 258, 243 and 36 gp120 Env-reactive memory B-cells, respectively. Approximately 50% of cells isolated from each animal bound to all Env immunogens. Conversely, 12%-21% bound to a single immunogen. Antibodies from memory B-cell culture supernatants that bound to CH505.TF infected cells were identified from all animals, but CH505.TF V2-specific antibodies only from one NHP (10/258 supernatants). In contrast, AE.A244 V2 cross-reacting antibodies were frequent (7%-13%; 3/3 NHPs). While ADCC-mediating antibodies against CH505.TF were more frequent (6%-12%) than against UMMS B, no ADCC-mediating antibodies targeted V2 peptides. **Conclusions:** This regimen focused the memory B-cell response on epitopes shared among the four immunogens. Memory B-cells producing antibodies that bound or mediated ADCC on CH505.TF-infected cells were elicited. However, they were not directed to V2, and CH505.TF V2-reacting memory B-cells were induced only sporadically. Epitope focusing and lack of cross-reacting ADCC-mediating V2 responses against the challenge virus may have contributed to the inability of this regimen to protect NHPs from challenge.

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Therapeutic Vaccination Induces Antiviral T Cell Response in SIV-Infected ART-Treated Infant Macaques

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Globally, 2.1 million children are living with HIV-1 and the majority of new infections occur postnatally through breast milk transmission. While antiretroviral therapy (ART) has improved disease outcome and reduced transmission, residual immune activation persists during ART and interruption of ART leads to rapid viral rebound due to the latent viral reservoir. Interventions to delay or prevent viral rebound in the absence of ART would be highly beneficial to the pediatric population who must remain on ART throughout their lifespan. In this study, two doses of a therapeutic vaccine, recombinant adenovirus serotype 48 (Ad48), was administered to SIV-infected ART-treated infant rhesus macaques (RMs) and the antiviral immune response was evaluated. Sixteen 4-week-old RMs were infected with SIVmac251 orally in two doses 24 h apart and placed on daily ART beginning at 4 wks post infection. A 3 to 5 log reduction in plasma viral loads was observed prior to vaccine regimen. Eight RMs received two doses of Ad48 at 22 and 30 wpi. The remaining eight RMs were used as experimental controls. Complete blood count (CBC), serum chemistry, plasma viral loads, and T cell kinetics were monitored throughout the study. IFN-g ELISPOT responses to SIVmac239 Gag, Pol, and Env peptide pools were evaluated prior to vaccination and 4 wks post-second Ad48 and Gag-specific CD8+ and CD4+ T cell responses were measured by multiparametric intracellular cytokine staining (ICS) for TNFa and IFNg. This treatment protocol was tolerated in the infant RMs with no adverse clinical observations and normal CBC and chemistry values throughout the study. The SIV-specific T cell response was increased following vaccination, evident through both ELISPOT and ICS assays. In summary, we have demonstrated that Ad48 vaccination induces a strong anti-SIV T cell response in SIV-infected, ART-treated RMs. These results provide preclinical data for potential pediatric HIV cure strategies.

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Dynamics of peripheral blood cell subsets in newborn and young adult rhesus macaques following pathogenic SIV infection and combination antiretroviral therapy

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Background: The rhesus macaque model for HIV infection has been instrumental for prophylactic and therapeutic vaccine research and for testing immunologic interventions designed to improve virologic control following discontinuation of combination antiretroviral therapy (cART). The immune systems of these outbred animals manifest substantial diversity before infection according to genetics, age, and presence of subclinical viral infections such as cytomegalovirus. Comprehensive analyses of the cellular dynamics of innate and adaptive immune components in peripheral blood can identify profiles associated with the outcome of infections and interventions. **Materials and Methods:** Cells in whole blood were stained with a panel of 18 monoclonal antibodies including CD3, CD4, CD8, CD11b, CD11c, CD14, CD16, CD20, CD25, CD28, CD80, CD94 (NKG2A), CD95, CD123, CD127, CD185 (CXCR5), CD279 (PD-1), and HLA-DR. White blood cells (WBC) were analyzed by multiparameter flow cytometry after lysis and fixation. The assigned animals included 2-week-old newborns (*n*=14), 10-15-month-old specific pathogen-free (SPF) infants (*n*=20), and 3-year-old SPF young adults (*n*=12) before and after SIVmac251 infection and initiation of cART. **Results:** By combining one-step flow cytometry and electronic cell counting, we developed a strategy to quickly and precisely determine the phenotype of between 93.3-99.1% (median 97.7%) of WBC in peripheral blood of 46 healthy macaques. Principal component analysis of over 50 variables derived from the assay clustered animals from the same age group very closely, demonstrating age-related changes in profiles of mononuclear cells. The alteration of blood-cell composition after pathogenic SIVmac251 infection and administration of cART will be reported. **Conclusions:** Information obtained by this comprehensive analysis of the composition and phenotype of mononuclear cell subsets is useful for (1) identifying unique signature profiles associated with experimental outcomes and (2) evaluating the evolution of macaque cell profiles in experimental trials. This project is supported by awards from the National Institutes of Health (AI143554 and AI131568).

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A CCR5-blocking antibody for HIV pre-exposure prophylaxis

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In the absence of a prophylactic vaccine, the use of antiretroviral medications as pre-exposure prophylaxis (PrEP) to prevent HIV acquisition by uninfected individuals is a promising approach to slowing the epidemic. Unfortunately, negative side effects, viral resistance, and lack of regimen adherence severely limit PrEP efficacy. Thus, new approaches are urgently needed. The use of the CCR5 coreceptor by mucosally transmitted virus, together with the high resistance to sexually transmitted HIV observed in CCR5D32 homozygous individuals, indicates that CCR5 blocking reagents might be effective novel PrEP agents. Leronlimab, formerly PRO140, is a CCR5-specific humanized IgG4 monoclonal antibody currently in clinical trials for use as HIV monotherapy with FDA approval expected in 2020. Using a panel of CCR5- and CXCR4-tropic viruses *in vitro*, we demonstrated that Leronlimab fully prevented infection of human and macaque CD4+ T cells by CCR5-tropic viruses. In macaques infected with CCR5-tropic $SHIV_{SF162P3}$, once-weekly subcutaneous Leronlimab monotherapy achieved a 4-log reduction in plasma viremia. We observed no depletion of CCR5+ cells or downregulation of CCR5 on CD4+ T cells in Leronlimab-treated animals. Rather, Leronlimab fully occupied CCR5 receptors on CD4+ T cells isolated from all tissues examined, including sites of mucosal HIV transmission such as rectum and vagina. Based on these results, we initiated a PrEP study to test the ability of Leronlimab to prevent acquisition of $SHIV_{SF162P3}$ following low dose, repeated intra-rectal challenges. At the abstract deadline, we have performed eight challenges that resulted in the infection of all five control animals, while all three Leronlimab-treated animals are protected. We are currently enrolling additional animals into the study protocol and will present the latest results. Cumulatively, our results establish Leronlimab as a novel reagent for PrEP and cure studies where mimicking the homozygous CCR5D32 phenotype is advantageous.

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High titer, multi-target serum neutralizing antibody responses are associated with protection against autologous challenge in BG505 SOSIP immunized rhesus macaques

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Stabilized HIV-1 envelope (Env) trimer immunogens consistently elicit autologous neutralizing antibody responses that have been associated with protection. Two groups of rhesus macaques were immunized with BG505 SOSIP.664 trimer in 3M-052 adjuvant. One group received only the SOSIP while the other group also received viral vectors expressing SIVmac239 Gag. Both immunization regimens provided significant protection against vaginal challenge with SHIV.BG505. High serum autologous neutralizing antibody titers on the day of challenge were associated with protection, most notably in the SOSIP only group. Neutralizing antibody epitopes were mapped using serum from 12 protected monkeys and a panel of BG505 Env pseudovirus glycosylation mutants affecting 4 defined neutralizing antibody targets: V1 residues 133/136, 241/289 and 465 glycan holes, and a neoepitope proximal to N611 at the trimer base. Neutralizing antibodies in the protected monkeys commonly targeted the 465 and 241/289 glycan holes. In contrast, the V1 epitope was targeted in only one monkey. Neutralization was frequently enhanced when the N611 glycan was removed. Further negative stain EM studies using serum IgG from both protected and infected monkeys revealed multiple binding sites on BG505 SOSIP.664, a subset of which overlapped with the neutralization targets. A monoclonal antibody with neutralizing activity against the autologous Env was recovered from a protected monkey whose high titer serum neutralizing activity was targeted predominantly against the 465 glycan hole. Mapping studies to determine whether the monoclonal antibody also targets the 465 glycan hole epitope are underway. Overall, the data supports that glycan holes and epitopes near the trimer base are commonly targeted in BG505 SOSIP immunized monkeys. Moreover, simultaneous recognition of multiple targets could contribute to high neutralization titers and protection against autologous challenge.

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Parallel effects of RhCMV or HIV infection on the intestinal microbiota

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The gut microbiota and chronic viral infections both profoundly influence the host immune system, but interactions between these influences have not been explored. Cytomegalovirus (CMV), for example, infects 50-90% of the human population and drives significant changes in immune-cell phenotypes and functions. Similarly, certain members of the commensal gut microbial community affect T-cell development in mice, macaques, and non-human primates. However, the complex interplay between viral infections, microbiome composition, and host immunity is not fully elucidated. It is unknown if some immunologic effects of chronic viral infection could be secondary to changes imposed on the host intestinal microbiome. Here we show that rhesus cytomegalovirus (RhCMV) infection in rhesus macaques is associated with specific differences in gut microbiota composition, including decreased abundance of Firmicutes and increased abundance of Bacteroidetes. Furthermore, RhCMV infection diminished associations seen in seronegative animals between specific bacterial taxa in the gut and immune-cell phenotypes, suggesting that CMV infection asserts a far greater influence than gut microbes. Similar changes were seen in a cohort of seronegative animals that were vaccinated with RhCMV-vectored SIV vaccines. Significantly, *Streptococcus*, the only bacterial genus that increased in RhCMV infected rhesus macaque, has been shown to induce IL-10 production in the gut, further suggesting that IL-10 signaling pathway have an important role in the pathogenesis of RhCMV. Additionally, bacterial genera *Faecalibacterium* and *Blautia* were also decreased in HIV infected individuals, suggesting that similar metabolic changes might have occurred. These data suggest that CMV modulates the relationships between gut microbiota and host immunity.

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Genetic and phenotypic changes in SIV sooty mangabey virus during its evolution towards HIV-2

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Human Immunodeficiency Virus Type 2 (HIV-2) is thought to have originated from an ancestral simian immunodeficiency virus native to sooty mangabeys (SIVsm). Following the initial cross-species transmission and subsequent spread, the progenitor virus is believed to have undergone numerous adaptive changes and gained virulence causing immunosuppressive disease in humans. However, the details on the evolution of both the intermediate and culminating genetic changes remain unknown. In this regard, humanized mice provide an attractive *in vivo* model to simulate the cross-species transmission and viral evolution. Hu-mice develop a full complement of human immune cells, which include human T and B cells, monocytes/macrophages, and dendritic cells and thus are permissive for HIV infection. Here, we infected hu-HSC mice with the progenitor SIVsmE041 virus and sequentially passaged it for 8 generations spanning nearly four years to mimic the natural transmission of the virus to humans and its subsequent spread. Infection kinetics during each passage were monitored for plasma viral loads and $CD4^+$ T cell decline. Viral genetic changes during each serial passage were assessed by next-generation sequencing (NGS) of the entire viral genomes. Upon initial infection with the parent virus, plasma viremia was detected within 3 weeks which remained persistent. Discernable $CD4^+$ T cell decline was also noted. The viral set points and the levels of $CD4^+$ T cell decline increased over time indicating viral adaptation and increased virulence. Viruses obtained from plasma at multiple time-points within a given passage/generation were assessed for single-nucleotide variants utilizing NGS. Several amino acid substitutions were identified throughout the genome that were non-synonymous and became fixed in genes such as *gag, pol,* and *env*, indicative of cross-species viral adaptation. This data validates the important role that humanized mice can play in modeling the viral evolution of animal viruses that can infect humans and give rise to epidemics.

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The effect of depot medroxyprogesterone acetate on TAF metabolism in rhesus macaques

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Background: Prevention of HIV infection and unintended pregnancies are public health priorities. In sub-Saharan Africa, where HIV prevalence is highest, depot-medroxyprogesterone acetate (DMPA) is widely used as contraception. Therefore, understanding potential interactions between DMPA and antiretrovirals is critical. We evaluated the effect of DMPA on the pharmacokinetics (PK) and tissue distribution of tenofovir alafenamide (TAF) in macaques. **Methods:** The PK profile of TAF was evaluated in female rhesus macaques receiving a single human-equivalent dose of oral TAF (1.5 mg/kg). Macaques received DMPA (30mg,intramuscularly) 11 days before TAF dosing (n=9) or were untreated controls (n=8). Tenofovir (TFV) and TFV-diphosphate (TFV-DP) were measured in PBMCs, rectal fluids (R_F), vaginal fluids (V_F) and tissues. **Results:** The median area under the curve (AUC_{0-72h}) values for TFV-DP in PBMCs were similar in DMPA-treated (6,991 fmol*h/10~6 cells) and untreated controls (5,256 fmol*h/10~6 cells) (p=0.15;t-test). Plasma TFV was <40ng/ml in both groups, and only detected 1h after TAF dosing in 3/17 animals. Median TFV AUC_{0-72h} values in R_F were also similar between DMPA-treated and untreated animals (1,070 and 868 ug*h/ml, respectively;p=0.702). In rectal tissues TFV-DP was only detected in DMPA-treated macaques [median=20.9 (4.9-91.2 fmol/mg tissue)]. TFV was undetectable in V_F from all DMPA-treated animals, but was consistently detected in the untreated controls, albeit at low concentrations (median AUC_{0-72h} = 27.4 ug*h/ml). None of the DMPA-treated or untreated animals had detectable TFV-DP in vaginal tissues. **Conclusions:** We show high-dose DMPA treatment in macaques has no systemic effect on TFV-DP in PBMCs or TFV in plasma, which is relevant for HIV treatment. However, DMPA was associated with increased TFV-DP in rectal tissues. The low TFV-DP in vaginal tissues may have important implications for efficacy of PrEP with single-agent TAF products. Additional dose-ranging studies are needed to define mucosal TFV and TFV-DP steady-state levels and confirm effects of DMPA treatment.

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Focusing humoral responses to V2 region of HIV Env by DNA priming vaccination with trimeric V1V2 immunogen

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Background: RV144 clinical trial showed that non-neutralizing antibodies targeting the V1V2 region of HIV gp120 correlated with reduced risk of infection, making this region an important vaccine target. To induce V1V2-specific antibodies, we tested the immunogenicity of a vaccine regimen that includes priming with DNA expressing scaffolded trimeric V1V2. **Methods**: We compared two vaccine regimens, including 2 DNA priming followed by 2 DNA+protein co-immunization boosts, for the induction of antibodies targeting the V1V2. Macaques (N=4/group) were primed with A244 $V1V2_{2J9C}$ DNA expressing soluble trimeric V1V2 (V1V2 group) or A244 gp145 DNA (gp145 group) that expresses membrane-bound trimeric Env and soluble gp120. The booster vaccine in both groups comprised A244 gp145 DNA and gp120 protein adjuvanted in GLA-SE. The V1V2 primed group also received $V1V2_{2J9C}$ DNA. Antibodies were monitored after the prime and the boost. **Results**: V1V2 primed animals developed more V1V2-binding antibodies compared to animals in the gp145 group, while both regimens induced robust antibodies recognizing gp120. The V1V2 DNA vaccine also induced higher antibody responses to cyclic V2 peptides from different clades (B, C, E). The antibodies also recognized trimeric Env anchored on the cell surface. Peptide mapping showed greater breadth of Ab responses within the V2 region in the V1V2 primed group. The DNA+protein co-immunization boost increased antibody responses in both groups. Magnitude and breadth of the V2 responses remained higher in V1V2 group. Responses to the V2 peptide RDKKQKVHALFYKLDIVPIE (HXB2 AA 166-185), a critical target identified in RV144, were found only in the V1V2 group. **Conclusions**: Our results demonstrate that priming with DNA expressing trimeric V1V2 immunogen focuses the responses to the V1V2 region of gp120 with cross-clade specificities. This regimen alters the immune-hierarchy of the antibodies induced by Env, providing a headstart for antibodies to V1V2. This vaccine regimen could be more effective in reducing infection risk.

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Developing a robust pregnant rhesus macaque model to test Zika virus treatments and vaccines

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One in seven women infected with Zika virus (ZIKV) during pregnancy will have a baby born with Congenital Zika Syndrome. However, there are no FDA approved treatment options for pregnant women diagnosed with ZIKV. Animal models play a crucial role in developing treatments and vaccines for use during pregnancy considering the increased perceived risk of testing these options in pregnant women. We developed a pregnant rhesus macaque model to study the pathophysiology of ZIKV, however, adverse outcomes in this model were not sufficient to study efficacy of a treatment to prevent neonatal injury. We initially used an Asian-lineage ZIKV isolated from a Puerto Rican patient (ZIKV-PR) inoculating macaques with 10~4 plaque-forming units (pfu), a dose mirroring that administered by a mosquito. However, adverse fetal outcomes were not consistent in this model. In this study, we infected three pregnant macaques with 10~8 pfu of an African-lineage virus (ZIKV-Dak). Two of three animals had a productive infection with peak viral loads greater than 5x10~5 vRNA copies/ml. One of the two animals with productive infection had a fetal demise at gestation day 59, 17 days post-infection. There was detectable ZIKV in all fetal and maternal/fetal interface tissues tested suggesting ZIKV was the likely cause of the demise. The other animal with productive infection is currently still pregnant. Preliminary results from this study suggest that a supraphysiological dose of African-lineage Zika virus may have led to increased adverse fetal outcomes in at least one of two animals. Infection of additional animals with this dose and strain are necessary to fully understand the rate of adverse fetal effects from this model relative to our current model. If it results in more severe outcomes, this model system may allow for more robust testing of ZIKV treatments in the future with fewer animals.

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Pre-existing SIVmac239 infection alters the immunological response to M. tuberculosis infection in a Mauritian Cynomolgus macaque model of SIV/Mtb co-infection

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Mycobacterium tuberculosis (Mtb) is the most common cause of morbidity and mortality in HIV+ individuals. However, it is not well understood how HIV infection alters the immunological response to Mtb, leading to loss of bacterial containment. We previously established a Mauritian cynomolgus macaque (MCM) model of SIV and Mtb co-infection. We found that SIV+ MCM challenged with a low dose (~10 CFU) of Mtb Erdman exhibited more rapid granuloma dissemination between 4 and 8 weeks post infection, compared to SIV-naïve MCM that were similarly challenged with Mtb. This observation led to the hypothesis that SIV infection disrupts the early host immune response to Mtb, thus weakening the ability of the host to control the mycobacterial infection. To test this hypothesis, we challenged SIV+ and SIV-naïve animals with a low dose of Mtb Erdman, and euthanized the animals at 6 weeks post infection. At time of necropsy, there were no marked differences in the TB disease burden between the two cohorts. However, SIV+ animals had many more CD8 T cells in BAL following Mtb coinfection. Furthermore, flow cytometric analyses of individual granulomas and affected lymph nodes revealed that the CD4/CD8 T cell ratio was lower within affected tissues of SIV+ MCM compared to SIV-naïve MCM. CD4 and CD8 T cells in SIV+ MCM expressed increased levels of the activation/exhaustion markers PD1 and TIGIT. Additionally, there was less expression of the cytokine TNF in the CD4 and CD8 T cells in SIV+ MCM, compared to those who were SIV-naïve. Overall, we identified that while TB disease was not different after 6 weeks of Mtb infection between the two groups, immunologic differences were identified and may explain why SIV+ animals are eventually unable to contain Mtb and rapidly develop TB.

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Identification of viral reservoirs despite early ART in SIV-infected rhesus macaques

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Whereas antiretroviral therapy (ART) suppresses viral replication, ART discontinuation results in viral rebound, indicating the presence of viral reservoirs (VRs) established within lymphoid tissues. Herein, by sorting myeloid and CD4 T cell subsets of SIVmac251-infected rhesus macaques (RMs), we demonstrated that effector memory (TEM) and follicular helper (TFH) $CD4^+$T cells are major VRs in the spleen and mesenteric LNs of ART-treated RMs. These VRs are established despite ART administrated at day 4 post-infection. We also demonstrated that myeloid cells, which included classical (CD14+), intermediate (CD14+CD16+), and non-classical (CD16+) cells can be infected early after viral exposure expressing both viral DNA and RNA in the the spleen. In contrary to CD4 T cells, early ART drastically controls myeloid infection. Finally, after ART interruption, we demonstrated the rapid seeding of SIV in all the tissues supporting the role of these T cell subsets in viral rebound. Altogether our results contributed in our understanding of early viral seeding in which visceral lymphoid tissues are crucial in maintaining VRs

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Optimization of Fixation Methods for Flow Cytometry of Non-Human Primate Lymphocytes

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Background: High-parameter flow cytometry is increasingly used in non-human primate (NHP) research because large amounts of data can be collected from limited sample. Flow cytometry acquisition cannot always occur immediately after staining cells, so researchers often fix after staining to delay acquisition. There is no consensus about which protocol and buffer is optimal for stability of fluorochromes over time. Standardization is critical, because fixation can degrade certain fluorochromes, increase autofluorescence, and worsen spectral overlap, potentially compromising the integrity of data. We investigated the optimal fixative, method, and length of storage for generating sensitive, reproducible, high-quality data. **Methods**: Three fixation methods were tested on peripheral blood mononuclear cells (PBMC) from rhesus macaques using BD Stabilizing Fixative: 1) fix and leave in fixative, 2) fix overnight and resuspend in PBS, and 3) fix for one hour and resuspend in PBS. We tested five fluorochromes and acquired the data 1, 2, 5, and 9 days post-fixation. Fluorochromes tested were Pacific Blue, PE, PE-Cy7, APC-Cy7, and BV650. Data from 13 fluorescent channels were collected, and compensation matrices, spectral overlap, fluorescent intensity, and sensitivity were analyzed using FlowJo v10.4. **Results**: Fixing cells without washing resulted in decreased fluorescent intensity of PE within 24 hours, which continued to decrease dramatically over a period of 9 days. Non-specific binding of BV650 and Pacific Blue significantly increased after washing at all time points resulting in false positives. The fluorescent intensity and sensitivity of PE-Cy7 and APC-Cy7 were unaffected by washing method or duration of fixation. **Conclusions**: Fixation methods impact quality of flow cytometry data. We found that the optimal method of fixation varied by fluorochrome, thus, great care should be taken when selecting a method for fixation and storage, with consideration for the fluorochromes being used in the panel.
Funding was provided by NIH/NIAID HHSN272201700022C and P51 OD011104

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NIAID Reagent Resource Support Contract for AIDS Vaccine Development – "Gene to Protein" method for large-scale production of monoclonal antibodies

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The NIAID Reagent Resource Support Contract for AIDS Vaccine Development (Contract Number: HHSN272201800007C) was established to support research leading to the development of vaccines, therapeutics, and medical diagnostics for the prevention, treatment, and diagnosis of infectious and immune-mediated diseases. Through Quality Biological (QBI), the program provides high-quality, novel, and targeted reagents, assays and services for investigators engaged in AIDS vaccine-related research. The program supplies investigators with critical reagents involved in AIDS vaccine studies and development. QBI provides a repository for these reagents with the appropriate storage and shipping conditions to maintain their stability and integrity. The QA/QC of these reagents and services assures consistency while affording a level of standardization among laboratories. As an example, QBI is collaborating with the NIAID Vaccine Research Center (VRC) to produce gram amounts of 4 anti-SIVenv monoclonal antibodies, ITS54, ITS55, ITS58, ITS67 to be used in a series of prospective NHP passive immunization SVEU studies. Those antibodies, together with a control antibody (anti-Zika) were produced in single-use bioreactors by transient transfection in Expi293 mammalian cells to display "average glycan content". The purification was performed by affinity chromatography on a recombinant A resin. The final product was sterile filtered and assessed for protein concentration and purity, endotoxin measurement and mycoplasma detection.

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Intracellular antiretroviral drug levels in CD4+ follicular helper T cells in SIV-infected rhesus macaques on combination antiretroviral therapy (cART).

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Background: A main barrier to HIV cure is persistence of the latent virus in immune privileged sites. In particular, CD4+ follicular helper T cells (Tfh) resident in lymph node (LN) B cell follicles (BCF) have been reported as a major cell compartment contributing to viral persistence. While there is relative exclusion of cytotoxic CD8+ T cells in BCF, it is possible that restricted drug penetration into BCF can also contribute to reservoir persistence. To test this possibility, we compared ART levels in Tfh and non-Tfh of SIV-infected rhesus macaques (RM) on cART. **Methods:** Five chronically SIVmac239-infected RM received subcutaneously administered cART consisting of tenofovir (5.1mg/kg), emtricitabine (40mg/kg) and dolutegravir (2.5mg/kg), daily for seven days. Then, RM were euthanized and major lymphoid tissues were collected. In addition, axillary LN (AxLN), mesenteric LN (MesLN) and spleen were sort-purified to isolate CD4+ Tfh and non-Tfh cells. Liquid chromatography-triple-quadrupole mass spectrometry was used to quantify intracellular drug levels in tissues and sorted cells. Plasma viral loads (pvl) were quantified by RT-PCR. **Results:** cART resulted in a 3-log reduction in pvl by time of necropsy (Geomean 397,428 to 664 copies/ml). While there were different patterns of drug distribution among tissues, concentrations of all three drugs were consistently lower in secondary lymphoid tissues and gut compared to PBMC. In sorted subsets, Tfh had tendency of slightly lower concentration of intracellular tenofovir-diphosphate (TFV-DP) than non-Tfh (median value of fmol/10^6 cells in Tfh vs. Non-Tfh: 1299 vs. 1555 in AxLN; 707 vs. 1204 in Mes.LN; 718 vs. 1406 in Spleen). **Conclusions:** We observed lower concentrations of cART drugs in secondary lymphoid tissues compared with blood. TFV-DP distributes into Tfh in secondary lymphoid tissues with some limited penetration. These data suggest that HIV/SIV persistence in Tfh is a complex interplay between drug biodistribution and immune-mediated mechanisms.

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Functional Analysis of Rhesus Macaque Fcgamma Receptors

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The rhesus macaque has become an indispensable animal model for the preclinical evaluation of immunotherapies and vaccines to combat HIV-1; however, polymorphisms in macaque Fcg receptors (FcgRs) that are not present in humans can complicate the interpretation of Fc-mediated antibody responses. To assess the functional effects of these polymorphisms, luciferase reporter cell lines expressing common alleles of human and rhesus macaque FcgRs were incubated with B cells in the presence of anti-CD20 antibodies representing each of the four subclasses of human and rhesus IgG (hIgG1-4 and rIgG1-4). FcgR-mediated responses were measured as the upregulation of luciferase over a range of antibody concentrations and EC_{50} values were calculated. Five alleles of FcgRIIa and three alleles of FcgRIIIa were selected to represent the coding polymorphisms present in the majority of Indian-origin rhesus macaques. All were functional, but differed in their capacity to interact with human versus rhesus macaque antibodies. In contrast to human FcgRIIa, rhesus FcgRIIa variants exhibited strong responses to rIgG1 and rIgG2 as well as detectable responses to rIgG3. Furthermore, unlike human FcgRIIa, four of the five rhesus FcgRIIa variants responded better to rIgG2 than to rIgG1. In the case of FcgRIIIa, all three rhesus variants preferentially responded to IgG1 with similarly low EC_{50} values for hIgG1 and rIgG1. However, these receptors differed dramatically in their interactions with the other IgG subclasses. Whereas responses to hIgG2-4 were barely detectable, the rhesus FcgRIIIa variants all exhibited strong responses in the presence of rIgG2, rIgG3 and rIgG4. These results reveal species-specific differences in the functional interactions of antibodies with human and macaque FcgRs and provide an important foundation for investigating FcgR-mediated mechanisms of protection in the rhesus macaque.

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High-throughput identification of MHC class I binding peptides using an ultradense peptide array

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A successful HIV vaccine will likely need to elicit both anti-HIV antibody and CD8 T cell responses. SIVmac239-specific CD8 T cell epitopes restricted by just five rhesus macaque MHC alleles have been thoroughly defined, limiting the animals that can be used in SIV studies and creating a knowledge gap regarding the role of specific MHC alleles in SIV control and progression. However, conventional methods for CD8 T cell epitope identification are labor-intensive and do not scale well. We accelerate this process by using an ultradense peptide array as a high-throughput tool for screening peptides to identify putative novel epitopes. In a single experiment, we assess the binding of four Indian rhesus macaque MHC class I molecules – Mamu-A1*001, -A1*002, -B*008, and -B*017 – to 61,066 8-mer, 9-mer, and 10-mer peptides derived from the full proteomes of 82 SIV and SHIV isolates. Many epitope-specific CD8 T cell responses restricted by these MHC molecules have already been identified in SIVmac239, providing an ideal dataset for validating the array; up to 64% of these known epitopes are found in the top 192 SIVmac239 peptides with the highest MHC binding signals in our experiment. To assess whether the peptide array could identify putative novel CD8 T cell epitopes, we performed IFN-γ ELISPOTs and found three novel peptides that elicited CD8 T cell responses in cells from at least two Mamu-A1*001-positive animals; two of these were validated by *ex vivo* tetramer staining. This high-throughput identification of peptides that bind class I MHC will enable more efficient CD8 T cell response profiling, allowing us to rapidly characterize the SIVmac239 peptide binding repertoires of additional rhesus MHC alleles, and to identify CD8 T cell epitopes in other pathogens with more complex proteomes that interact with HIV/SIV, such as *Mycobacterium tuberculosis*.

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Twenty-eight-color phenotyping of rhesus macaque lymphocytes

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In order to identify multiple cellular phenotypes in samples from non-human primates several staining panels are generally required, which results in excess time and a copious amount of sample used. By using a strategic antibody cocktail and a highly sensitive flow cytometer, throughput was increased and the sample size used was decreased, improving efficiency. With the use of a BD FACSymphony configured to analyze up to 30 parameters, we were able to demonstrate the ability of an optimized 28-color panel to identify subsets of T cells, B cells, NK cells and dendritic cells. The panel incorporated cell specific markers along with exclusion markers to remove cells that are not of interest. With the use of the FlowJo application, individual subsets were characterized after gating on live CD45+CD14- singlet cells. With this panel design we were able to identify multiple subsets of T cells which included Th17 (CCR6+), Th2 (CXCR3- CCR6-), Th1 (CXCR3+), and Tfh (CXCR5+ PD-1+) cells. Subsets of T cell memory and NK T cells (CD3+ NKG2a/c +) were also identified in this panel. B cells subsets analyzed in this panel included naïve mature (CD21+CD27-IgD+), unswitched memory (CD21+CD27+IgD+) and swithched memory (IgD-IgM-). NK cells in this panel were identified as CD3-CD20- NKG2a/c+, CD16+ and CD56+. Dendritic cells were characterized as CD3-CD20-CD19-CD20- and pDCs can be distinguished as CD123+ or CD123- while mDCs are phenotipically characterized as CD11c+ cells. Overall, this broad phenotyping flow panel design and flow cytometric analysis promoted efficiency by saving time and conserving the amount of sample used in the experiment. From this study, similar panel design could provide for more efficient and broad cellular phenotyping in the future.

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Neuroinflammation and CNS HIV-1 establishment following mucosal SHIV transmission in rhesus macaques

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HIV+ patients are at an alarmingly higher risk of developing HIV associated neurocognitive disorders (HAND). HAND is a spectrum of neurocognitive deficits linked to impairments in the prefrontal cortex (PFC) and hippocampus, areas controlling higher cognitive processes. Studies in HIV+ patients implicate acute viremia and inflammation in driving central nervous system (CNS) impairment. However, the timing and implications of viral dissemination to the CNS, particularly the PFC and hippocampus, remain undefined. We tested the hypothesis that HIV-1 is rapidly and actively established within areas of the CNS responsible for controlling cognition. Here, we demonstrate HIV-1 dissemination within the CNS following vaginal transmission of the T cell tropic transmitted/founder SHIV.C.CH505. Strikingly, despite moderate systemic viremia (PVL range:200-22,000 cp/ml), we found evidence of local HIV-1 replication within the cerebrospinal fluid (CSF) (23 - 900 cp/ml); an observation substantiated by HIV-1 within the choroid plexus. Presence of viral RNA and pro-viral DNA in the brain suggested active influx of infected CD4 T cells and/or monocytes into the CNS. Correspondingly, virus was disseminated within multiple brain regions including PFC, hippocampus, and superior temporal sulcus (STS). In further support of active CNS immune influx, CSF levels of interferon protein 10 and interleukin 6, central drivers of neuroinflammation, were markedly elevated. Presence of IgG against HIV-1 envelope in the CSF reflected equilibration between the systemic and CNS compartments during acute infection. In affirmation of these findings, high-resolution confocal microscopy showed blood-brain barrier disruption in multiple brain regions including: PFC, hippocampus, and STS. Notably, activated microglia and astrocytes, colocalized with p27 HIV-1, and surrounded disrupted blood vessels. Together, our data show rapid HIV-1 dissemination into the PFC and hippocampus following vaginal transmission. Acute HIV-1 establishment within the CNS has significant implications for functional neurological deficits in HAND and lays the foundation to identify mechanisms underlying HAND neuropathogenesis.

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Spleens of rapidly progressing SIV+ infant macaques reveal elevated type I interferon responses and suppressed B cell replication

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The spleen has an essential role in mounting humoral immune responses, however the impact of HIV/SIV infection on the spleen is largely unknown. We utilized an SIV-infected infant rhesus macaque model to evaluate spleen-associated humoral immune dysfunction. Twenty-five infant macaques ranging from 2 to 4 months of age were orally infected with SIVmac251 and followed longitudinally for 10 to 17 weeks. We assessed germinal center development in spleens via immunofluorescence (IFA) staining for CD20 and Ki-67 followed by quantification in Imaris software by measuring Ki-67+ cells per follicle area. Infants that progressed rapidly to disease – as defined by low/undetectable levels of SIV-specific serum IgG – possessed significantly fewer actively replicating splenic germinal centers (CD20+, Ki67+) than typical progressors. To assess SIV infected cells and cell-free virus within splenic B cell follicles, we utilized in situ hybridization (RNAscope) with SIV-RNA specific probes; rapid progressing infants exhibited more SIV+ cells proximal to germinal centers in splenic white pulp. We hypothesized that type 1 interferon responses were negatively influencing the replication status of the B cells in the germinal centers; to evaluate this, we IFA stained for CD20 and Mx1, an interferon regulated protein. Mx1 response was found to be elevated in the splenic B cell follicles of rapid progressors versus typical progressors. These data provide evidence that rapid progression to AIDS in infant macaques is associated with presence of virus-associated cells, follicular type-1 interferon levels, and failure to induce Ki67+ germinal centers. Understanding splenic dysfunction therefore provides important clues as to how SIV infection can ultimately augment antibody responses in SIV/HIV infected hosts.

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Visualization and characterization of systemic, mucosal and lymphoid granulocyte populations in rhesus macaques

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Granulocytes are essential for immune protection due to their ability to destroy pathogens by phagocytosis, NETs, or degranulation, and stimulate immune cell recruitment Neutrophils, for example, have been shown to prevent in vitro HIV infection by netosis in the female genital tract However, limited research describes the role of granulocytes in HIV/SIV infections and mucosal immunity Our goal was to understand their roles in lentivirus infection particularly in mucosal tissues of naïve and infected rhesus macaques Mucosal tissues (jejunum, colon, cervix, vagina), lymph nodes, spleen, liver, and whole blood were collected from naïve, SIV+, and SHIV+ rhesus macaques Cells were characterized using flow cytometric staining for surface markers- CD14, CD123, CD49d, CD66, CD16, and FcRE- after gating on live CD45+CD3-CD20- cells, Fc receptors – CD64, CD32, CD16 and FcRe, and trafficking/activation markers - CD11b, CD62L, CD63 and CD69 Imagestream flow cytometry was used to confirm granulocyte phenotype using surface marker staining, nuclear visualization with DAPI, and brightfield images, with data density analyses using IDEAS software We identified phenotypes of granulocyte subsets as- CD45+CD66+CD49d+ eosinophils, CD45+CD66+CD14+ neutrophils, and CD45+CD123+FcRE+ basophils Polynuclear and mononuclear visualization with DAPI staining and surface marker images by Imagestream analysis confirmed granulocytic phenotypes Flow cytometry data showed that differential expression of FcRgs can be helpful in distinguishing granulocyte subsets while FcRe was only expressed by basophils Interestingly, frequencies of granulocyte populations were generally higher in mucosal compared to lymphoid tissues in both naïve and infected animals Eosinophils were particularly enriched within jejunal tissue, with a median of 45% of CD45+ cells We were able to differentiate granulocyte populations in whole blood and tissues of rhesus macaques High frequencies in mucosal tissues indicate importance of gut and reproductive tract immunity Overall, more investigation of infected tissues is needed to understand the significance of granulocytes in mucosae and infection.

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CytoDRAV: Dimensionality Reduction and Visualization for Flow Cytometry in Rhesus macaques

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Technical advances in flow cytometry have quickly enabled researchers to generate high-dimensional datasets containing millions of data points. Traditional analyses of these data utilize biaxial plots and manual gating around populations of interest. Dimensionality reduction algorithms, such as PCA and t-SNE, are a promising avenue to supplement traditional analyses by allowing researchers to visualize multidimensional data in a 2-dimensional space. Our laboratories have recently developed CytoDRAV which is a graphical interface to several powerful open-source R packages – flowCore, Rtsne, and ggplot2 – that allow researchers to load FCS files, tune t-SNE parameters, and visualize t-SNE outputs in an easy-to-use and highly customizable fashion. Here, we demonstrate the specific applicability of CytoDRAV and up to 28-color cytometry to study difficult to define nonhuman primate lymphoid populations using rhesus macaque samples and pre-processed files gated for live CD45+ cells, T cells, B cells, monocytes, and NK cells. Further, these analyses identify minor or novel subpopulations of lymphocytes including natural killer-like B cells (NKB), NKT cells, and other regulatory and innate T cell populations. Using CytoDRAV as a highly sensitive biomarker discovery approach could have broad applicability for predicting even very subtle disease-relevant changes in macaque models (i.e., SIV).

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SIV infection alters viral kinetics and immune responses during ZIKV co-infection in female pigtail macaques

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Background: Globally over 36 million people are infected with HIV and Zika virus (ZIKV) outbreaks have occurred in geographic areas endemic with HIV infection. Currently, there is a severe lack of knowledge regarding the risks of ZIKV co-infection in people living with HIV. Understanding whether HIV co-infection alters ZIKV pathogenesis will be important when evaluating ZIKV vaccines and therapeutics, especially in high-risk groups such as HIV-infected pregnant mothers and infants. Here, using SIV-infected pigtail macaques, we investigated the hypothesis that enhanced ZIKV pathogenesis occurs in people living with HIV. Material & Methods: Female pigtail macaques (n=3) were infected with SIVmac239M and co-infected with ZIKV at 9 weeks post-SIV infection ($SIV^+/ZIKV^+$). Animals were compared to contemporary/historical control females (n=7) only infected with ZIKV ($SIV^-/ZIKV^+$). SIV and ZIKV viral loads in plasma and peripheral tissues were measured by qRT-PCR. Blood/PBMCs, lymph node, and mucosal tissues (rectum, vagina) collected pre- and post-SIV and/or ZIKV infection were evaluated for innate and adaptive immune responses by flow cytometry. Markers of microbial translocation and inflammation in plasma and cerebral spinal fluid were measured by ELISA and multiplex assays. Results: ZIKV viremia in $SIV^-/ZIKV^+$ control animals was detected 2-7 days post-infection (dpi), a pattern that is consistent with previous reports in the pigtail model. In contrast, ZIKV viremia was detected for a longer duration (2-10 dpi) in $SIV^+/ZIKV^+$ animals. Post-ZIKV infection, recruitment of CD16+ peripheral monocytes was dampened and delayed in $SIV^+/ZIKV^+$ vs $SIV^-/ZIKV^+$ animals, corresponding with the delayed ZIKV viremia in $SIV^+/ZIKV^+$ animals. Markers of microbial translocation in the blood increased post-ZIKV in $SIV^+/ZIKV^+$ but not in $SIV^-/ZIKV^+$ animals. Analyses of ZIKV burden and adaptive immune responses in tissues are ongoing. Conclusions: We provide evidence that immune responses against ZIKV are delayed and dampened in SIV-infected macaques and SIV infection may lead to altered ZIKV pathogenesis.

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A novel vaccine targeting the viral protease cleavage sites protects Mauritian cynomolgus macaques against vaginal SIVmac251 infection

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After over three decades of research, an effective anti-HIV vaccine remains elusive. Unconventional and novel vaccine strategies are needed. Here, we report that a vaccine focusing the immune response on the sequences surrounding the 12 viral protease cleavage sites (PCSs) provides greater than 80% protection of Mauritian cynomolgus macaques (MCMs) against repeated intravaginal SIVmac251 challenges. The PCS-specific T cell responses are correlated with vaccine efficacy. The PCS vaccine does not induce immune activation and inflammation known to be associated with increased susceptibility to HIV infection. Machine learning analyses revealed that the immune environment generated by the PCS vaccine predicts vaccine efficacy. Our study demonstrates for the first time that a novel vaccine which targets viral maturation, but lacks full Env and Gag proteins as immunogens, can prevent intravaginal infection in a highly stringent NHP/SIV challenge model. Targeting HIV maturation thus offers a novel approach to developing an effective HIV vaccine.

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Characterization of SHIV.CH505 immunopathogenesis in rhesus macaques

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Background: The majority of simian-human immunodeficiency viruses (SHIVs) currently available have significant limitations in that they were created using HIV-1 Env sequences from chronically HIV-infected individuals, uncommon HIV subtypes, or were optimized by serially passaging *in vitro* or *in vivo*. Recently, the newly developed SHIV.CH505, which incorporates the *vpu-env(gp140)* sequence from a transmitted/founder HIV-1 subtype C strain (CH505) was shown to retain attributes of primary HIV-1 strains. Here, we characterize the immunopathogenesis of SHIV.CH505 in mucosal tissues of rhesus macaques. **Methods:** Male rhesus macaques (n=7) underwent multiple low-dose intra-rectal challenges with SHIV.C.CH505. Viral challenge was halted when animals tested PCR positive for viral sequences in plasma. Colon and rectum biopsies were collected pre- and post-infection and used to monitor intestinal immune populations. **Results:** All animals became productively infected within 6 challenges and exhibited similar acute viral replication kinetics, including a median peak viral load of $1x10^6$ RNA copies/ml plasma (range=$0.89x10^6$–$5.5x10^6$) reached by two weeks post-infection. Set point viral loads ranged from $3.8x10^3$–$0.99x10^6$ RNA copies/ml plasma. By week2-post-infection, we observed significant and persistent depletions of CCR5+ and CCR6+ CD4+ T cells in mucosal tissues, decreases in CD4+ T cells producing Th17 cell-associated cytokines, CD8+ T cell dysfunction and alterations of B cell and innate immune cell function. **Conclusions:** In line with previous findings, we demonstrate that SHIV.CH505 is capable of infecting and replicating efficiently in rhesus macaques after low-dose intra-rectal challenge, resulting in peripheral viral kinetics similar to that seen in SIV/HIV infection. Furthermore, our findings indicate that this virus is capable of eliciting intestinal immunopathology typical of SIV/HIV. These findings affirm the value of this novel SHIV as a robust tool that can be used in a variety of applications, including preclinical testing of new therapies or prevention strategies.

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Developing an SIV in vitro latency model by directly infecting primary resting rhesus macaque CD4+ T cells

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Antiretroviral therapy (ART) durably controls human immunodeficiency virus (HIV) infection. However, the presence of a transcriptionally silent reservoir of latently infected cells that can resume replication in the absence of ART impedes the development of a functional cure. It is currently unknown how far the reservoir must be reduced to achieve long-term remission. In this study, we aim to investigate this by determining the relationship between the number of latently infected cells and the time until viral rebound after ART interruption. To accomplish this, we are developing a unique two-step approach to artificially generate a latent simian immunodeficiency virus (SIV) reservoir in rhesus macaques. Our goal is to produce latently infected cells *in vitro* that recapitulate the resting $CD4^+$ T cell reservoir *in vivo*. First, $CD4^+$ T cells are isolated from SIV-naïve rhesus macaques and made permissive by incubating with cytokines/chemokines (CCL19, IL-2, IL-7, or IL-15) or a FOXO1 transcription factor inhibitor (AS1842856) for three days. Next, the cells are infected with SIVmac239 and cultured with antiretrovirals. At five days post-infection, the infection rate is determined by stimulating the cells with anti-CD3/CD28-coated beads and measuring intracellular SIV Gag by flow cytometry. Using this approach, we achieve infection rates of 0.1-0.3% of $CD4^+$ T cells. Importantly, unstimulated cells maintain a resting state and express low levels of T cell activation markers (CD25, HLA-DR). Once fully established, this *in vitro* latency model will enable the autologous transfer of latently infected cells into SIV-negative macaques under the cover of ART, producing artificial latent reservoirs of known magnitude. By manipulating the reservoir's size in non-human primates, this approach has the potential to define the minimum number of latently infected cells necessary for viral recrudescence and sensitively detect changes in the reservoir after therapeutic interventions.

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Pharyngeal motor cortex grey matter abnormalities and retinal photoreceptor layer dysfunction in macaques exposed to Zika virus in utero

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One third of infants who have prenatal Zika virus (ZIKV) exposure and lack significant defects consistent with congenital Zika syndrome (CZS) manifest neurodevelopmental deficits in their second year of life. We hypothesized that prenatal ZIKV exposure would lead to brain abnormalities and neurodevelopmental delays in infant macaques, as measured by quantitative hearing, neurodevelopmental, ocular and brain imaging studies. We inoculated 5 pregnant rhesus macaques with ZIKV during the first trimester, monitored pregnancies with serial ultrasounds, determined plasma viral RNA (vRNA) loads, and evaluated the infants for birth defects and neurodevelopmental deficits during their first week of life. ZIKV-exposed and control infants (n=16) were evaluated with neurobehavioral assessments, ophthalmic examinations, optical coherence tomography, electroretinography with visual evoked potentials, hearing examinations, magnetic resonance imaging (MRI) of the brain, gross post mortem examination, and histopathological and vRNA analyses of approximately 40 tissues and fluids. All 5 dams had ZIKV vRNA in plasma and seroconverted following ZIKV inoculation. One pregnancy resulted in a stillbirth. The ZIKV-exposed infants had decreased cumulative feeding volumes and weight gains compared with control infants, and also had grey matter abnormalities in the pharyngeal motor cortex identified by quantitative voxel-based morphometric comparisons. Quantitative ocular studies identified differences between ZIKV-exposed and control infants in retinal layer thicknesses and electroretinograms that were not identified in qualitative ophthalmic evaluations. Despite these findings of neuropathology, no ZIKV vRNA or IgM was detected in the infants. This suggests that ZIKV exposure without measurable vertical transmission can affect brain development in utero and that subtle neurodevelopmental delays may be detected with quantitative analyses in early infancy. Quantitative brain analyses, such as these, may predict neurodevelopmental delays that manifest later in childhood and allow early intervention and targeted therapies to improve functional outcomes of ZIKV exposed children.

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Increased risk of early cytomegalovirus (CMV) infection in SIV-infected infant rhesus macaques

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Although AIDS-related mortality due to emergence of cytomegalovirus (CMV) as an opportunistic infection has greatly declined since the advent of potent antiretroviral treatment (ART), CMV co-infection continues to be a significant contributor to co-morbidities in HIV-infected individuals on ART. CMV co-infection is also a poor prognostic factor in pediatric HIV infection, which is highly prevalent in Western and Central Africa. While maternal HIV infection increases the risk of congenital CMV infection, less is known about the risk of postnatal CMV acquisition and its effect on pediatric HIV infection in the absence or presence of ART. We investigated the risk of postnatal CMV acquisition in a cohort of neonatal and infant SIV-infected rhesus macaques that were separated from their mothers within 7 days of birth (mean 2 days; range 1-7 days) and SIV-infected soon thereafter (n=9 neonates) or at 5 months of age (n=6 infants). While maternal CMV antibodies detected at birth waned to undetectable levels, three of 9 SIV-infected neonates on ART and one of 6 SIV-infected infants on ART progressed to AIDS within four months of infection and had detectable plasma CMV DNA in the absence of CMV seroconversion near end-point. Overall, postnatal CMV infection detected in 27% of SIV-infected macaques (4 of 15) contrasted with 0% detection in a cohort of 59 SIV-negative rhesus macaques separated from their mothers within 72 hours of birth and followed for more than one year (P-Value 0.0012; Fisher's Exact test). In a 15-month longitudinal follow-up of three SIV-negative infants paired with their CMV-seropositive mothers, plasma CMV DNA followed by seroconversion was detected in only one infant after 9 months despite daily exposure to maternal CMV shed in urine, saliva and breast milk. These data suggest that HIV-infected infants are at increased risk of earlier acquisition of CMV infection and accelerated AIDS progression.

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CD8B+ and Cytolytic CD4+ T cells Contribute to Spontaneous Post-Treatment Control of SIV Infection

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Current combination antiretroviral therapy (cART) is effective at suppressing plasma viremia in HIV-infected individuals, however, plasma viral loads rebound upon cART interruption. Recrudescence of replication-competent virus following cART interruption necessitates lifelong treatment with cART. Post-treatment controllers (PTCs) i.e. HIV-infected individuals that control plasma viremia after cART interruption may inform the development of therapeutic strategies to induce off-cART virologic remission. Results from cohorts of PTCs determined that early cART initiation, small reservoir size and the presence of polyfunctional HIV-specific $CD4^+$T cells are factors that mediate post-treatment control. There is a need to further understand the underlying mechanisms driving virologic control in these individuals. Non-human primate (NHP) models of AIDS are potentially powerful tools for identifying immunological correlates and mechanisms for PTCs that may be challenging to identify in humans. Here we explore plausible cellular immune responses contributing to the control of SIVmac239 in rhesus macaques that exhibit prolonged viral control upon cART interruption. Our data show that the antigen-responding $CD137^+$ $CD8^+$ T cells are more cytolytic and exhausted in PTCs compared to non-controllers (NC). We demonstrate that *in vivo*depletion of $CD8b^+$T cells results in a transient increase in plasma viremia, suggesting they play a role in viral control in PTCs. Intriguingly, virologic control was reasserted without recovery of the depleted $CD8b^+$T cells. Our results suggest the role of another cell type, cytolytic $CD4^+$T cells, in maintaining virologic control in PTCs. Cytolytic $CD4^+$T cells in PTCs were characterized as $CD137^+CD107a^+GranzymeB^+$with upregulated exhaustion marker expression. We also report a higher frequency of cytolytic $CD4^+$T cells in PTCs compared to NCs. Our next steps include identifying the epigenetic mechanism of action of $CD8b^+$and cytolytic $CD4^+$T cells in mediating post-treatment control of SIV infection.

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OPTIMAL MATURATION OF SIV-SPECIFIC CD8+ T CELLS IS ASSOCIATED WITH NATURAL CONTROL

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Background: Highly efficient CD8⁺ T-cells are associated with spontaneous HIV/SIV control, but it remains unclear how and when these cells are generated. **Methods**:We monitored for 18 months cynomolgus macaques (n=16) naturally controlling viremia (SIV controllers-SICs) or not (viremics-VIRs). The development and evolution of CD8⁺ T-cell responses were assessed functionally and phenotypically. **Results**:Polyfunctional SIV-specific CD8⁺ T-cells emerged during primary infection in both SICs and VIRs. Neither the magnitude nor the polyfunctional profile of these CD8⁺ T-cells correlated with SIV control at any time. In contrast, the functional ability of CD8⁺ T-cells to suppress SIV replication *ex-vivo,* which was low in early stages, increased after a period of maturation of several weeks in SICs, leading to the establishment of sustained low-level viremia (e.g., r=-0.55,p=0.03, CD8-SIV suppression day 70 post-infection[p.i.] *versus* viral load). The divergent evolution of the SIV suppressive capacity of CD8⁺ T-cells in SICs and VIRs was associated with early differences in the phenotype of their SIV-specific CD8⁺ T-cells. Central memory (CM) CD8⁺ T-cells from SICs displayed higher levels of CD127 since day 14p.i. In contrast, a persistently skewed differentiation phenotype of CM CD8⁺ T-cells, typified by high levels of T-bet, was observed in VIRs since primary infection. Negative correlations were observed between T-bet expression in CM SIV-specific CD8⁺ T-cells and CD8-SIV suppression at acute (r=-0.48,p=0.06) and chronic (r=-0.68,p=0.005) SIV-infection, while levels of CD127 expression correlated positively with CD8-SIV suppression (acute:r=0.5,p=0.05; chronic:r=0.48,p=0.05) and negatively with viral loads (acute:r=-0.59,p=0.02; chronic:r=-0.67,p=0.004). **Conclusions**:The capacity of CD8⁺ T-cells to suppress SIV infection characterized natural control. Importantly, this activity was not immediately acquired by SIV-specific CD8+ T-cells but was gained overtime in SICs, in relation to the early establishment of appropriate CM response in these animals. Conversely, SIV-specific CD8⁺ T-cell responses in VIRs failed to gain antiviral potency due to early defects imprinted in the CM pool.

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DNA/MVA Therapeutic Vaccination Induces Robust Polyfunctional SIV Specific- T Cell Response and Antigen Recognition Breadth in Chronically SIVmac239 Infected Rhesus Macaques Under ART

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HIV/SIV infections are characterized by dysfunctional antiviral immunity and persistent viral reservoirs. Combination Anti-retroviral therapy (ART) is effective in controlling the virus replication but does not significantly improve the function of anti-viral immunity and/or reduce the viral reservoirs. Given the high mutation rate of HIV/SIV, the breadth of anti-viral T cell response is also important. Thus, there is a great need for the development of therapeutic vaccines that can induce high magnitude and broad anti-viral CD4 and CD8 T cell response with improved function. Towards this goal, we employed a CD40L plus TLR7 agonist -adjuvanted DNA/MVA SIV vaccine in SIV-infected rhesus macaques (RMs). Six SIVmac239 infected ART suppressed RMs received two DNA vaccinations followed by two MVA vaccinations 4 to 8 weeks apart and another group of six received no vaccination. The DNA vaccinations induced low levels of SIV Gag and Env specific IFNg+ CD4 and CD8 T cell response. Following the 1st MVA boost, we observed a strong induction of SIV-specific IFNg+ CD4 (up to 1%) as well as CD8 (up to 6.7%) T cell response. These cells were highly polyfunctional and co-expressed other cytokines TNFa and IL-2. The SIV-specific CD8 T cells also expressed high levels of perforin and granzyme B indicating their killing potential. Furthermore, vaccination significantly enhanced the breadth of CD4 (recognized 10-21 pools, average of 14) and CD8 (3-11 pools, average of 6.5) T cell response. In addition, we observed high levels of SIV-specific CD8 T cells in extra-follicular and follicular regions of lymph node sections following MVA boost using IHC. These data show that therapeutic DNA/MVA vaccination can induce a high magnitude of virus-specific CD4 and CD8 T cells with polyfunctionality, breadth and potential to home to B-cell follicles. ART interruption is in progress to study the influence of vaccination on viral control.

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Classical MHC-I restricted rhCMV-specific immune responses

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Our understanding of rhesus cytomegalovirus (rhCMV)-specific cellular immune responses has reached an extraordinarily exciting era several years ago. Directing the spotlight to CD8 T cells that are restricted by Mamu-E MHC-I antigen presenting molecules all, but made us overlook CMV-specific CD8 T cells, that recognize their epitope in the context of classical MHC-I molecules. In the absence of basic tools, we are unable to address fundamental questions. Such as the role of classical and non-classical CD8 T cell responses in the life-long control of CMV-infection. To that end, we screened PBMC from 74 rhesus macaques of Indian origin to find IFN-γ responses after stimulation with pools of peptide 15-mers covering the entire length of rhCMV IE-1 and pp65-2 proteins. We defined two epitopes, restricted by the Mamu-A1*002:01 and Mamu-A1*008:01 MHC-I molecules respectively. Since the Mamu-A1*002:01 allele frequency is close to 20% and the Mamu-A1*008:01 frequency is close to 30% in the Wisconsin National Primate Research Center colony, these two alleles enable us to monitor rhCMV responses in a significant portion of our animals. We detected rhCMV IE-1 Mamu-A1*002:01 epitope VY9 (VTTLGMALY aa291-299) tetramer positive T cells in 11/12 animals (median 1.45% of CD3+CD8+ cells, range 0.30-16.55). Interestingly, but not unexpectedly we found that the VY9 tetramer also bound to NK cells in 8/12 animals (median 6.65 of CD3-CD8+ cells range 2.02-21.7). We found that >85% of Mamu-A1*008:01 allele positive animals harbor response to the pp65-2 epitope NP8 (NPTDRPIP aa96-103) (median 1.26% of CD3+CD8+ cells, range 0.73-15.3). We are convinced that these reagents will be of benefit to the nonhuman primate research community both in the AIDS and non-AIDS research areas.

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Using RNA-flow to monitor alternative gene splicing in rhesus macaque NK cells

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Background: Infection/disease state can broadly affect gene expression including biasing some gene isoforms over others via alternative splicing. Measuring alternative splicing *ex-vivo* has been traditionally limited to techniques like RT-PCR and RNA-Seq and remains significantly under explored as a form of immune regulation, particularly in innate immunology. In this proof-of-principle study we used RNA-flow technology to characterize NK cells from rhesus macaques during SIV infection, and simultaneously quantify differences in gene splice variants following infection. **Methods**: Longitudinal peripheral NK cells from rhesus macaques infected with SIVmac239 (n=6) were sorted and RNA-Seq performed using an Illumina platform. RNA-Seq data was aligned using STAR aligner and Tuxedo suite was used for quantification of reads. To determine alternatively-spliced transcripts the data was aligned with salmon and alternative transcripts were quantified and annotated using the IsoformSwitchAnalyzeR package. Custom RNA-flow probesets targeting exon regions of the genes of interest were designed to quantify isoforms found in parallel RNA-Seq samples. **Results**: Rhesus NK cells were identified and sorted using a standard flow cytometry gating strategy: CD3-CD14-CD20-NKG2a/c+. Using traditional RNA-Seq data analyses we identified several groups of genes associated with normal NK cell antiviral responses, including IFN, cell cycle and mTOR-associated genes. Using the IsoformSwitchAnalyzeR package revealed an even more restricted gene-set of alternatively spliced genes. Utilizing RNA-flow we validated altered activation and transcriptional regulatory genes (i.e., HES4, CYLD and HARS) in NK cells following SIV infection that were unchanged in other immune cells. **Conclusions**: HIV/SIV infections result in still under-characterized immune regulation and/or dysfunction. Understanding how viral infection alters NK cell splicing in particular may help in devising more robust therapeutics harnessing NK cells against HIV and SIV, or other reconstitution modalities to combat immune exhaustion.

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Identification of a soluble factor inducing CXCR5 expression on NK cells

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B cell follicles are a major viral reservoir in HIV/SIV infections. Strikingly, African green monkeys (AGM) display a strong, NK-cell mediated viral control in follicles. During chronic SIVagm infection in AGM, NK cells from secondary lymphoid organs (lymph nodes, spleen) frequently express CXCR5 in contrast to NK cells from blood and gut of SIVagm-infected AGM and to NK cells from macaques. We aimed to identify the mechanism responsible for CXCR5 expression on NK cells. We collected blood and lymph nodes from six AGM before and during chronic SIVagm.sab92018 infection. Autologous NK and B cells were isolated with magnetic beads and cultured in RPMI-FCS (supplemented with IL-2/-4/-15) either alone or together with and without contact (in transwells). The phenotype of B and NK cells was determined by flow cytometry. RNA-seq method was applied on sorted $CXCR5^-$ and $CXCR5^+$ NK cells to determine the expression pattern of transcription factors and signaling pathways. The spatial dynamics of $CXCR5^+$ NK cells in tissues was analyzed through staining by spin disk and confocal microscopy. We observed an early NK cell migration into follicles during the acute phase of SIVagm infection (day 9 post-infection). We identified contacts between NK and B cells on tissue sections and in culture. The NK-B cell coculture in transwells as well as supernatants of B cells alone induced CXCR5 on NK cells. Analyses of the transcriptome data suggested a role of IL-6. IL-6 indeed induced in a dose-dependent manner CXCR5 on NK cells. Studies on B and NK cells from macaques are ongoing. Preliminary data show that IL-6 also upregulates CXCR5 on NK cells from healthy macaques. Our study reveals that IL-6 participates in the induction of CXCR5 on NK cells in AGM. Our results highlight an unprecedented crosstalk between NK and B cells.

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Lymph node-based CD3+CD20+ "double positive" cells results from trogocytosis between follicular helper T-cells and B cells and expands following SIV infection in rhesus macaques

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$CD4^+$ T follicular helper cells (T_{FH}) support the development of class-switched, affinity-matured antibodies by promoting germinal center B-cell responses. Tfh cells have also been indicated as one of the key cellular targets for HIV/SIV replication and persistence. In this study, we describe a novel $CD3^+CD20^+$ "double positive" (DP) lymphocyte cell subset resident in lymph nodes (LN) and spleen of rhesus macaques (RMs). The presence of CD3 and CD20 on the same cell was demonstrated by using a combination of flow cytometry, image stream analysis, and immune-histochemistry. $CD3^+CD20^+$lymphocytes (i) are enriched in cells showing T_{FH} phenotype ($CD4^+PD1^{bright}CXCR5^+ICOS^+Bcl\text{-}6^+$), function ($IL\text{-}21^+IL\text{-}17^-IL\text{-}2^{+/-}IFN\text{-}g^-$) and gene expression profile, and (ii) express other B-cell markers such as CD21, HLA-DR, CD79, and surface immunoglobulins. We also observed that expression of CD40L upon brief in vitro stimulation with PMA/Ionomycin identifies DP cells that are T_{FH} in origin vs. those of B-cell lineage based on specific gene-expression signatures. Cross-sectional and longitudinal analysis in 56 RMs, including 9 SIV- and 47 SIV+ pre- and on-ART, revealed an expansion in the frequency of DP cells among SIV+ as compared to SIV- RMs ($p=0.007$). Furthermore, DP cells are reduced after 12 months of ART when compared to the same animals before ART initiation ($p=0.002$) and expand again to be significantly higher as compared to SIV- once ART is interrupted ($p=0.003$). Altogether, our data suggest that (i) DP cells arise in secondary lymphoid tissues as a result of membrane exchange between T_{FH} and B-cells; (ii) The DP phenotype may identify a subset of T_{FH} and B-cells that have recently undergone high affinity interactions; and (iii) DP cells expend in vivo following SIV infection and replication. Further research is warrant to determine whether the expansion of DP cells impacts on antiviral immune responses, HIV pathogenesis, or HIV persistence. Supported by R01-AI-116379

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A combination of stabilized C.1086 clade C envelope trimer and clade B gp120 trimer protein boosts induce homologous and heterologous tier 2 neutralizing antibodies in macaques.

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Protein based vaccines induce strong antibody response. Previous studies have shown that boosting DNA/MVA vaccinated animals with unstablized clade C gp140 envelope (Env) protein fails to induce autologous and heterologous neutralizing antibodies and provide protection in macaques. Here we developed and boosted DNA/MVA-SHIV vaccinated rhesus macaques with a C.1086 gp140 uncleaved prefusion-optimized trimer protein (UFO.v2.HS_{RQY}) that was designed to match the clade C consensus sequence in the V2 hotspot (V2HS), enhance binding to broadly neutralizing antibodies, and form stable trimers. Animals were additionally boosted with a clade B JR-FL CycP gp120 trimer that induces a broad cross-clade V1V2 response. Two protein boosts were delivered subcutaneously with Alum-3M052 as adjuvant. Protein boosts induced robust binding antibody responses against both C.1086 and JRFL proteins. Importantly, 3 out of 10 animals developed a strong autologous neutralizing antibody titer (100 to 800). Impressively, all animals demonstrated a heterologous neutralizing antibody response against the hard to neutralize SHIV1157ipd3N4 Env G13.10. Antibodies in vaccine sera also showed strong binding to membrane anchored SHIV1157ipd3N4 Env G13.10 and they mediated ADCC against SHIV1157ipd3N4 infected cells. Preliminary data showed that vaccine sera bind to multiple membrane anchored heterologous tier 2 Envs from a global virus panel. These data show a combination of UFO.v2.HS_{RQY} and JR-FL CycP-gp120 protein boosts induce homologous and heterologous neutralizing antibodies with heterologous ADCC activity. These animals soon will be challenged to determine the vaccine efficacy

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Detection of virus in the brain with acute SIV infection in rhesus macaque

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HIV enters the central nervous system(CNS) during acute infection, but early time points are difficult to study in humans. Experimental CNS infections in nonhuman primate models have generally used swarm SIV stocks, passaged neurotropic viruses, or with concomitant CD8 depletion, which often resulted in overwhelming encephalitis1-4. A recent study of SHIV infection analyzed virus and lymphocytes presences in the brain of rhesus macaques after 12 weeks of infection5. In this study, we aimed to determine if SIV was detectable in the CNS within 7 and 14 days after infection. Methods: 9 rhesus macaques were infected rectally with SIVmac239X. 4 monkeys were sacrificed on day-7 post-infection and 5 were on day-14. We performed RNAscope to detect SIV positive cells in the frontal cortex, basal ganglia and thalamus and immunohistochemistry to identify CD3, CD68 and CD163 positive cells in the brain. We also quantified viral DNA and RNA quantities in gross brain tissues. Results: Quantitative PCR consistently detected viral DNA/RNA copies in brain tissues as early as 14 days post-infection and in one animal at 7 days post-infection. RNAscope determination of SIV resulted in detection of virus at both 7 and 14 day post-infection, with the highest number of SIV-infected cells in basal ganglia followed by thalamus. There were no significant differences in the numbers of SIV -infected cells between days-7 and -14 of SIV infection. Co-labelling of SIV RNA probes and cellular markers for immune cells revealed that CD163 positive macrophages were involved in viral invasion of the brain. CD68 positive macrophages and CD3 T-cells were mostly detected along the blood vessels but occasionally also in the brain parenchyma. Conclusion: In an acute model of SIV infection, we showed that SIV can be detected in the brain as early as 7 days post-infection, and is carried by CD163 positive peripheral macrophages.

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Dynamics of CD8αβ and CD8αα cell populations in Rhesus Macaques

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In humans and nonhuman primates CD8 is expressed as a heterodimer of an alpha and beta chain (CD8αβ) or as a homodimer of two alpha chains (CD8αα). In rhesus, CD8αβ expression is limited to CD8+ T cells (T8) while CD8αα is expressed on many different leukocytes, including T8, CD4+ T (T4), B, NK and NKT cells. Although both form a complex with the T Cell Receptor (TCR) and MHC I, CD8αβ and CD8αα are not functionally equivalent in their actions. We have utilized an absolute cell counting flow cytometry assay to investigate these various populations in SIV-naïve, SIV-infected and in animals treated with either anti-CD8α or anti-CD8β depleting antibodies. We screened 37 healthy SIV-naïve rhesus macaques (24 female/13 male) and found that the majority of T8 cells are T8αβ (55 to 96%) with absolute counts ranging from 305 to 2256 cells/μL and the minority are CD8αα (3 to 44%) with absolute counts ranging from 36 to 595 cells/μL. As has been shown before, animals treated *in vivo* with anti-CD8α typically experience complete depletion of all T8 cells (in peripheral blood), as well as any T4, B, and NK cells that express CD8α, for 3-6 weeks post-treatment. After this time, T8 populations recovered, but not completely, resulting in an altered T4/T8 ratio. However, in animals treated *in vivo* with anti-CD8β, the depletion of peripheral T8αβ cells was nearly complete but there was little or no effect on T8αα or other cell types that express only CD8α. In addition, even 8 months post depletion, T8αβ cell numbers showed only minimal recovery and T8αα cell numbers only slightly increased. Interestingly, regardless of the CD8 antibody used for depletion, the relative proportion of CD8αβ to CD8αα cells was significantly altered and the majority of circulating T8 cells only expressed CD8αα. NCI Contract No. HHSN261200800001E.

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PET/CT scanning as a method to investigate the dynamics of the viral reservoir during antiretroviral therapy and viral rebound

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Human immunodeficiency virus (HIV) treatment with antiretroviral therapy (ART) suppresses viral replication and eliminating viremia. During ART, the viral reservoir persists in long-lived memory T-cells; however, viral rebound following the cessation of ART is a rapid process that is inconsistent with that population of cells. Here we explore the viral reservoir and the dynamics of viral rebound. Rhesus macaques were infected with a single high-dose challenge and 4 days later started on a six-month ART regimen. PET/CT scans using radioactive ^{64}copper-labeled antibodies against viral proteins revealed the locations of infected cells during the establishment of the viral reservoir, and throughout treatment. After ART conclusion, tissues were harvested at four, five, seven, and ten days. PET scans visualized foci of infection within tissues and we obtained sections for microscopy specifically containing infected cells. Using this method, infected cells were observed in several tissues for the entirety of ART and in the absence of viremia. Reservoir rebound was efficiently detected by PET/CT as early as four days post ART cessation and infected cells were found in PET/CT positive tissues validating the method. Importantly, rebound was most robustly detected by PET/CT in the same sites where signal was last detected after initiation of ART, and surprisingly, PET indicates a large concentration of viral envelope in heart tissues. Also unexpectedly, initial results from tissues harvested post-rebound reveal that most of the infected cells found in the small intestine, FRT, and colon are not T-cells. So far we have identified two morphologically distinct populations of infected cells and phenotyping is ongoing. These data suggest that the viral reservoir is not limited to a small population of long-lived memory T cells. The use of PET/CT to direct the collection of tissue samples was a powerful tool that significantly increased the efficiency and impact of this work.

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In vivo validation of the viral barcoding of SIVmac239 and the development of new barcoded SIV and subtype B and C SHIVs

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Genetically barcoded viral populations are a powerful tool for evaluating overall viral population structure as well as assessing the dynamics and evolution of individual lineages *in vivo* over time. Barcoded viruses are generated by inserting a small, genetically unique tag into the viral genome, which is retained in all progeny virus. We recently reported barcoding the well-characterized molecular clone SIVmac239, resulting in a synthetic swarm (SIVmac239M) containing approximately 10,000 distinct viral clonotypes differing only by a 34-base barcode that can be tracked using next generation deep sequencing. In this study, we assessed the population size, distribution, and authenticity of individual viral clonotypes within this synthetic swarm using samples from 120 rhesus macaques infected intravenously with a range of SIVmac239M inoculum doses. The number of barcodes in plasma correlated with infectious inoculum dose, and the primary viral growth rate was similar in all infected animals regardless of inoculum size. Overall, 97% of detectable clonotypes in the viral stock were identified in the plasma of at least one infected animal, demonstrating that only a small fraction of detectable clones may be nonfunctional. Importantly, minimal variability between the measured clonotype proportions was observed when replicate PCR and sequencing were performed. Additionally, we prepared a second generation SIVmac239 (SIVmac239M2), which contains over 16-times the number of barcoded variants of the original stock, and a barcoded stock of SIVmac239 with suboptimal nucleotides corrected to the optimal base (SIVmac239Opt5M). We also generated 5 novel barcoded viral stocks from subtype B and C SHIV clones. These new SHIV clones may be particularly valuable models to evaluate Env-targeting approaches to study viral transmission or viral reservoir clearance. Overall, this work further establishes the reliability of the barcoded virus approach and highlights the feasibility of adapting this technique to other viral clones.

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Increased in vivo persistence and activity of engineered rhesus macaque T-cells by minimizing ex vivo manipulation

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Adoptive T-cell immunotherapy holds great promise for treating many diseases. However, optimizing persistence, trafficking and in vivo activity of engineered cells remains a challenge. We developed a method to activate, transduce, and infuse autologous rhesus macaque primary lymphocytes transduced to express SIV-specific T-cell receptors (TCR) and homing markers in 4 days, from apheresis to infusion. Compared to methods involving longer and more extensive ex vivo manipulations this approach provided increased persistence, improved tissue homing, and enhanced activity of adoptively transferred engineered T-cells. In vivo expansion of infused cells was augmented by administration of recombinant rhesus heterodimeric IL-15 (Rhhet-IL-15) around the time of infusion. Previously, infused cells were detected for only 2-3 weeks post infusion with minimal expansion in vivo. Cells engineered using our new protocol are regularly detected 2 months or more after infusion and have expanded to as much as 20% of the total circulating CD8 compartment. The shorter engineering protocol reduces phenotypic changes typically seen in ex vivo preparation of engineered T-cells for adoptive T-cell therapy (ACT). Multiple rounds of ex vivo stimulation and extended culture skew the phenotype of engineered cells towards an effector memory profile with extensive pulmonary localization of infused cells. By minimizing ex vivo manipulation and time in culture, the ratio of effector memory to central memory T cells is maintained similar to the endogenous ratio seen in vivo in the cell donor, with decreased pulmonary trapping and an increase in effector memory cells circulating in PMBC and homing to target tissues. Initial studies using this improved method for T-cell engineering in SIV-infected rhesus macaques have shown promising antiviral effects. Contract HHSN261200800001E - Funded by the National Cancer Institute

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EFFECTS OF LONG-TERM RAPAMYCIN TREATMENT ON SIV PERSISTENCE IN RHESUS MACAQUES ON ANTIRETROVIRAL THERAPY

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Background: The mammalian target of rapamycin (mTOR) is a central regulatory kinase governing cell growth, proliferation, metabolism and effector functions in response to nutrients, stress and other stimuli. Inhibition of mTOR has been associated with altered immune function that may disrupt HIV persistence, such as: (a) limiting T cell proliferation, (b) reducing CCR5 expression on T cells, and (c) enhancing cytotoxic T cell responses. Here we evaluated the impact of long-term mTOR inhibition with rapamycin on T cell homeostasis, gene expression and viral reservoirs in SIV-infected rhesus macaques (RM) on combination antiretroviral therapy (cART). **Methods**: Adult male RM were intravenously inoculated with SIVmac239 before starting cART (tenofovir, emtricitabine and dolutegravir) 12 days post-infection. Following 219 days of cART, RM were randomized into 2 groups and given twice daily intramuscular injections of rapamycin at 0.02 mg/kg (n=7) or vehicle (n=7) for a total of 312 days. Lymphocyte populations were characterized by flow cytometry, plasma viral loads and cell-associated SIV RNA and DNA were quantified by qRT-PCR and qPCR, respectively, and changes in gene expression assessed by RNA-Seq. **Results:** Rapamycin induced a rapid and sustained increase in CXCR5 expression and a decrease in CCR5, Ki67 and HLA-DR on CD4+ memory T cells in rapamycin-treated RM versus controls. Gene expression pathways associated with T cell activation and metabolism were also significantly altered with rapamycin treatment. Despite these changes, SIV DNA and RNA in blood and lymph nodes remained stable over time with no significant difference observed between treatment groups. In addition, rapamycin had no effect on time to virus rebound or post-cART viral control following cART cessation. **Conclusions:** Collectively, these studies demonstrate that long-term rapamycin exposure induces significant changes in immune cell activation, proliferation and metabolism. However, rapamycin had no definitive effect on the stability of the SIV reservoir in RM on cART.

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Accumulation CXCR5+ NK cells in the lymph node during chronic SHIV infection is associated with enhanced viral control

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Natural killer cells (NKs) play an essential role in antiviral immunity; however, knowledge pertaining to their function in secondary lymphoid organs during chronic HIV/SIV infection is not fully elucidated. Lymph node (LN) follicles constitute major reservoir sites for HIV/SIV persistence. Cure strategies could benefit from the characterization of CXCR5$^+$NK cells able to access/eliminate HIV-reservoirs. Here we studied the phenotype, distribution and function of CXCR5$^+$ NK cells in the LN of SHIV naïve and chronic SHIV-infected (>14 weeks PI) rhesus macaques (RM) and their association with plasma viral RNA levels. We found that prior to infection, a significant proportion of NK cells (~15%) expressed CXCR5. Following infection, the frequency of CXCR5$^+$NK cells was significantly higher in chronic SHIV-infected RM. Phenotypically CXCR5$^+$NK cells express higher levels of FCgRIIa (CD32a) and FCgRIIIa (CD16) compared to CXCR5$^-$NK cells, which might be important for ADCC function. The CXCR5$^+$NK cells demonstrated enhanced polyfunctionality with higher production of IFN-g, TNF-aand CD107a when stimulated with mitogen. Transcriptional profiling (RNA-seq) of sorted CXCR5$^+$and CXCR5$^-$NK cells from LN of chronic SHIV-infected RM revealed that CXCR5$^+$NK cells are activated and express increased levels of cytolytic markers (perforin, granzyme-B, granulysin and CD107a), suggesting that these cells have a higher capacity to kill. Gene set enrichment analysis additionally showed elevated expression in CXCR5$^+$cells of transcripts associated with cell activation, TNF-alpha, and interferon signaling and apoptosis. Importantly, the frequency of CXCR5$^+$NK cells correlated inversely with plasma SHIV viral RNA levels and exhibited a significant negative association with germinal center Tfh cells. Thus, these data suggest that follicular homing NK cells could play an important role in controlling chronic SHIV infection. Cure strategies should focus on inducing CXCR5$^+$NK cells for sustained viral remission.

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Targeting RNA for HIV intervention

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Human Immunodeficiency Virus (HIV) is an RNA virus that integrates into the human genome and uses cellular machinery to replicate. It can also persist in a form of 'latent reservoir' hiding from complete eradication by the immune system or anti-retroviral drugs. Current drugs eliminate actively multiplying HIV but are unable to eradicate the latent virus. The latent virus can re-activate, and multiply. One treatment approach is to transplant HIV-1 resistant cells carrying silencing RNAs (siRNA) to destroy HIV. siRNAs bind to HIV RNA and trigger human cellular machinery to degrade HIV. However, HIV has evolved to readily inhibit or escape this machinery. To ensure long-term success of an HIV RNA targeting strategy, it needs to function independently of cellular machinery, and simultaneously target multiple conserved sites in the HIV RNA such that virus will be rendered non-functional if there are concurrent changes to the sequences in those sites. We used the recently discovered Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/Cas13 [CasRx] proteins, which are programmable RNA-guided ribonuclease targeting single-stranded RNA (ssRNA), reportedly independent of RNA structural constraints and showed fewer off-target effects. HIV-1 RNA could be efficiently targeted using CasRx protein in combination with poly-gRNA strings simultaneously targeting distinct conserved regions in HIV. The vector is small enough to be adapted to delivery through AAV. Thus results from this study can be easily adapted for translational applications. This approach has the potential to mediate long-term expression of a corrective payload that avoids permanent genetic modifications or frequent re-administration. We will further adapt this targeting strategy to non-human primate model of HIV infection to further test its efficacy.

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Long-term sterile immunity induced by an adjuvant-containing live-attenuated AIDS virus

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Previous studies have been performed using vaccine viruses attenuated by genetic disruption of key regulatory genes. The moderately attenuated prototypic vaccine strain SIVmac239Δnef has been used in most studies; however, it does not provide sufficient effects to prevent infection. Ag85B is one of the most dominant proteins secreted from most mycobacterial species. It has been shown to induce substantial Th1 cell proliferation and vigorous Th1 cytokine production. In the present study, we genetically constructed a live nef-deleted simian human immunodeficiency virus to express the adjuvant molecule Ag85B (SHIV-Ag85B) and assessed vaccine effects in cynomolgus macaques. SHIV-Ag85B could not be detected at 4 weeks after injection in cynomolgus macaques. When these macaques in which SHIV-Ag85B had become undetectable were challenged with pathogenic SHIV89.6P at 37 weeks after SHIV-Ag85B became undetectable, SHIV89.6P could not be detected at 36 weeks after the challenge in most of the macaques. In these macaques, SHIV antigen-specific $CD8^+$ T cell responses with polyfunctionality were rapidly induced after SHIV89.6P injection. Eradication of pathogenic SHIV89.6P was confirmed by adoptive transfer experiments and $CD8^+$ cell depletion study. These results suggest that SHIV-Ag85B elicited viral antigen-specific $CD8^+$ T cell responses against pathogenic SHIV and provide the possibility of eradicating a pathogenic lentivirus from the infected cells. The results of this study provide further insights into the containment of HIV infections as well as new opportunities to develop a better therapeutic vaccines and cure.

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NKTT320 mab-induced Natural Killer T lymphocyte activation promotes slower depletion of CCR5+ CD4+ T lymphocytes in acute SIV infection

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Invariant Natural Killer T cells (iNKT) are a subset of rapidly responding, evolutionarily conserved CD4+ T lymphocytes that express an invariant TCR capable of recognizing lipids presented on the non-polymorphic CD1d molecule. iNKT cells play a critical role in immune responses by bridging innate and adaptive immunity. iNKTs provide CD4 T helper function, cytotoxic effector functions, and modulate other innate and adaptive immune cells. Additionally, iNKTs are a primary target of HIV/SIV and are depleted and dysregulated early during infection. Treatment of Mauritian cynomolgus macaques (MCM) with a novel NKT-activating humanized monoclonal antibody, NKTT320, induces iNKT-specific activation and proliferation. In this study, we investigated the effects of NKTT320-induced iNKT activation on iNKT frequency and CD4+ T lymphocyte depletion in acute SIV infection. We performed a prospective longitudinal study of intrarectal SIVmac239 infection in four MCMs pre-treated with NKTT320 prior to SIV infection and compared them to four SIV-infected controls. A delay in detection of plasma SIV viremia was observed in the NKTT320-treated MCMs but peak plasma viremia at two weeks post infection was comparable in control and NKTT320-treated MCMs. In the first two weeks, a rapid and persistent decline in circulating CCR5+ CD4+ T lymphocytes was observed as early as one day post SIV infection in control SIV-infected MCMs, whereas CCR5+ CD4+ T lymphocyte depletion was first observed only at two weeks post SIV infection in the NKTT320-treated MCMs. A significant decline in blood iNKT frequency at two weeks post SIV infection was seen in control but not in NKTT320-treated MCMs. Overall, our preliminary data suggests that NKTT320 administration prior to SIV infection delays decline of target CD4+ T lymphocytes and iNKT in acute SIV infection. The long-term effect of these early beneficial differences on SIV infection outcome remains to be determined.

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Increasing SIV viral load assay sensitivity while maintaining high throughput and nominal sample volume requirements.

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Background: The Immunology and Virology Quality Assessment Center (IVQAC) at the Duke Human Vaccine Institute provides SIV viral load assays to Simian Vaccine Evaluation Units (SVEUs) as the contractor for the NIAD DAIDS Nonhuman Primate Core Virology Laboratory for AIDS Vaccine Research and Development. The standard high throughput SIV viral load assay performed at the IVQAC requires only 500 uL of plasma from which a limit of quantification (LOQ) of 62 RNA copies per milliliter is obtained. This standard assay meets the needs of the majority of NHP studies, however, there are certain types of studies that require the greatest sensitivity possible. In order to address this need, the IVQAC is developing an enhanced SIV viral load assay that, with only a modest increase in required plasma, significantly lowers the limit of quantification while still maintaining the high throughput of the standard assay.

Methods: Automated platforms are used for high throughput sample processing and PCR setup. Viral RNA is extracted from 1 mL of NHP plasma and eluted at 60 uL. Fifty microliters of the viral RNA is annealed to a SIV GAG specific reverse primer and reverse transcribed into cDNA. The cDNA is treated with RNase and then split between replicate 100 uL real-time PCR reactions containing target specific primers and a fluorescently labeled hydrolysis probes. Thermal cycling is performed on a real-time quantitative PCR (qPCR) instrument. Viral RNA copies per reaction are interpolated using quantification cycle data. Raw data is QC'd, positive and negative controls are checked and the mean viral RNA copies per milliliter are calculated.

An 8-member low-copy SIV viral load reference panel was created for the purpose of evaluating the limit of quantification for this enhanced SIV viral load assay. The panel was created by spiking negative NHP plasma with a known concentration of a highly characterized culture supernatant of SIVmac251 and then performing 1:2 serial dilutions to get viral loads ranging from 1000 RNA cp/mL down to 7 RNA cp/mL. This panel is currently being tested exhaustively to define the limit of quantification as well as other assay characteristics.

Results: Preliminary viral load results testing the low-copy SIV reference panel using the increased volume real-time PCR with twice the cDNA of the standard assay demonstrates increased sensitivity with detection in the 15-7 RNA copy per milliliter range. These results are promising but extensive testing will be required to confirm the limit of quantification on the enhanced assay.

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